AM
1937
ra

E74-21268

BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

MYOGENESIS OF THE CHICK

by

Beatrice Norma Rafter

(S.B., Massachusetts State College, 1936)

submitted in partial fulfilment of the

requirements for the degree of

Master of Arts

1937

1937
P2

TABLE OF CONTENTS

		page
I	Introduction	1
II	Early History	6
III	Establishment of the Mesoderm to Somite Formation	9
IV	Differentiation of the Primitive Segments	15
V	Histogenesis of Muscle	19
	1. Smooth Muscle	19
	2. Cross-striated Muscle	21
VI	Muscles of the Trunk	25
	1. The Deep Muscles of the Back	25
	2. The Thoraco-abdominal Muscles	26
	3. The Subvertebral Muscles	27
VII	Muscles of the Appendages	28
	1. Muscles of the Shoulder Girdle	31
	2. Muscles of the Thigh	35
VIII	Muscles of the Head and Neck	46
	1. The Laryngeal and Syringeal Muscles	52
	2. The Lingual Muscles	52
IX	Innervation	55
	1. Development of Nerves and their Innervation of the Muscles	57
	2. Development of the Spinal Nerves	60
	3. The Cerebral Nerves	64
	4. The Autonomic Nervous System	67
	Summary	72
	Bibliography	76

INTRODUCTION

In the year 1900, Charles Bardeen made the statement, "On no subject in vertebrate embryology is the literature less complete than that concerning the development of the voluntary apparatus." Most of the work that has been done in the study of muscle development was carried on during the early part of the twentieth century. Recent work has been more along the lines of experimental work to test the validity of past results, and also to determine the potencies of developing embryos when subjected to varying influences. However, Hunter, 1935, in his work with the hypoglossal musculature of the chick, and Romer, 1927, working on the thigh muscles have contributed considerably to the knowledge of the actual development and formation of the muscles of the chick. Since the greatest amount of work was done between 1900 and 1910, this paper will deal with the early embryologists only from an historical point of view, and concentrate on the later theories and experiments as they affect the developing musculature of the chick in an effort to bring together in one paper the results of many investigators.

Muscle tissue with the exception of a few muscles of the eye and some dermal muscles is of mesodermic origin and is best separated into three regions: the head, the trunk, and the limbs. The muscles of the head develop

directly from the mesoderm of the branchial arches and that in the dorsal eye region. The skeletal musculature, with the exception of that attached to the branchial arches, originates in the myotomes of the mesodermal segments of which the deep muscles of the back and the intrinsic thoraco-abdominal are the oldest phylogenetically and ontogenetically. Concerning the muscles of the limb buds there has been considerable dispute. At first it was believed that the limb muscles also were directly derived from the myotomes. Miss Byrnes, 1898, destroyed the myotomes in the leg region of amphibia and still obtained muscle formation in the injured leg. From these results she concluded that "the power to develop striated muscle is not restricted to the myotomes (mesothelium) but that the mesenchyme-like cells of the somatopleure can and do give rise to the voluntary muscles in the limb." Ingalls, 1907, observed cells from the ventral margins of the myotomes to lose connections with the myotome and become mingled with the mesenchyme of the limb bud. Later observers have never been able to obtain the same results, and favor the findings of Miss Byrnes.

It had been supposed that the first differentiation of muscles from mesoderm took place under the influence of the nervous system, and that self-differentiation did not occur. The views of the early embryologists disagree

considerably. But by 1900, Bardeen showed that in the pig embryo the musculature had differentiated considerably before the nerves established a connection with it, and concluded that the individual muscles were self-differentiating. Harrison, 1905-06, working with frogs, showed that when the nervous system was removed, the muscles showed differentiation absolutely independent of stimuli from nervous tissue. He also obtained the same results by suspending the action of the nerves with a 0.02 - 0.03 % acetone-chloroform solution. When the effect of the drug had worn off, the self-differentiated muscle was observed to function normally. Lewis, 1907, found that the muscle tissue is predetermined in the early gastrula of the frog. Hooker, 1911, found that there was development of functional voluntary and cardiac muscle of frog embryos without nerves, and Hoadley in 1925 found that muscle differentiation in the chick developed on the absence of any nervous influence.

It is probable that the formation of the definitive muscles is primarily due to the muscle mass, and secondarily to the relationship which the developing muscle has with the shifting skeletal elements. In the latter case, the developing muscles would be carried along with the shifting of the skeletal parts and are said to be migrating muscles. The origin of such muscles would be hard to trace

into the adult form if it were not for their innervation for the nerves indicate the paths along which the developing muscles migrated during their development. Thus it may be seen, in the early development, that a muscle mass arising in the lateral occipital region very early receives the extension of the nervus accesorius. Later in development toward adulthood, this nerve is found in the trapezius and sternocleidomastoideus muscles. The pectoralis major and minor arise in a cervical region and later extend into their adult positions while the rectus abdominis and other muscles of the abdominal wall shift from a lateral to a ventral position. There is a common facial muscle mass of the second branchial arch which is entered by the facial nerve before the muscle spreads to the head, face and neck. When this occurs the nerve also spreads with it. The muscle mass which is the anlage of the eye muscles is entered by the oculomotor nerve before the splitting into the definitive muscles takes place. The trigeminal nerve enters a common muscle mass in the mandibular arch which splits into the adult muscles which are supplied by that nerve. The lingual muscles have been found to originate from the two muscle masses early supplied by the two hypoglossal nerves, while the infrahyoid muscle comes from a mass supplied by the descending ramus of the hypoglossal. This gives an indication of the advantage of knowledge of

early innervation during the muscle formation. In some instances, a muscle is supplied by nerves from two or more segments which would suggest that such a muscle has a complex origin as in the case of the rectus abdominis and the obliquus internus and externus. However this does not always hold true because the muscle may receive other nerves secondarily and also retain the original nerve. This is shown quite readily in the trapezius muscle which later receives branches from the cervical plexus, and also by the digastric muscle which receives the mylohyoid nerve secondarily. "The site of entry of a nerve into a muscle, as a rule, marks the region of earliest differentiation" (Bardeen, 1907), and in many instances at least, "the distribution of the nerve within the adult muscle indicates the course of development or growth of that muscle." (Nussbaum, 1894) Before going on to a more detailed account of the early formation and later differentiation into the definitive musculature of the adult I will present an account of the beliefs and convictions of the embryologists in the period from 1855 to 1900.

EARLY HISTORY

Remak, 1855, produced the first consistent history of the somites of the chick. He believed that the somites originally were a hollow cubical mass of cells which, as the medullary groove deepened, gradually become triangular prisms with dorsal, medial, ventral and end walls. The medial edge elongated and divided into two leaf-like processes which united with the leaf-like processes of the opposite side to form the perichordal sheath or Wirbelkörpersäule. Also from the same mass a core of cells grew into the cavity. The core or Urwirbelkern fuses with all portions except the dorsal wall. The somite is now divided into an epitheloid upper wall (Rückentasel or Muskelplatte) and into a mesenchymal mass (Wirbelkernmasse). Remak also thought that the spinal nerves, ganglion and roots arose in the anterior portion of the mesenchymal mass.

His, 1868, said that each somite was primarily a flat quadrangular body consisting of a core and a cortex. The former consisted of a small cluster of irregular rounded cells, and the latter was composed of fusiform radiating cells which were attached to one another only at the base. The more posterior somites were more cuboidal and had larger cores than the anterior ones. He believed that the dorsal lamella was entirely converted into voluntary muscle.

His showed that the ganglia arose in the neural crest but he also believed that the core of the somite formed the sympathetic ganglia. The ventral wall of the somite formed only the muscular coat of the aorta.

Goette, 1875, pointed out that the tissues of the vertebral column came from the sclerotome (the Wirbelkern-masse of Remak).

Froriep, 1883, found a lyre-shaped mass of dense mesenchyme in each segment which extended from the mid-segmental point below the notochord laterally, dorsally and posteriorly to the following intersegmental fissure. This mass was later named the scleromere by Bardeen, and the entire segment was given the name of somite by Foster and Balfour.

Rabl, 1888, demonstrated that the dorsal lamella (His) or muscle plate (Remak) becomes a plate, the Hautmuskel-platte (dermomyotome) consisting of two layers. The inner layer, the Muskellamelle (myotome) only forms muscle, and the outer layer, the Cutislamelle (dermatome) forms the connective tissue of the dermis.

Patterson, 1888, found that the ventral edges of the dermomyotomes of the trunk of the chick grow downward into the membrane reuniens inferior and so supply the muscula-ture of the abdominal and thoracic walls, but that the musculature of the limbs does not arise directly from the dermomyotome.

Kaestner, 1890, opposing Patterson's view, believed that some of the cells of the cutis plate become myoblasts, and he also thought that the growing ventral edges of the dermomyotomes contributed directly to the limb muscles.

Kollman, 1891, worked with human embryos and claimed that the inner layer of the muscle plate gave rise to the dorsal musculature of the trunk, and the cutis plate gave rise to the musculature of the ventral part of the body and the limbs.

Fischel, 1895, thought that the growing edges of the dermomyotomes broke up into loose tissue which mingled with the mesenchyme of the nephrotome and the lateral plate. The mass of tissue formed in this manner then gave rise to the musculature of the limbs and the ventral parts of the body wall.

From the above views, it may be seen that there was general agreement concerning the myotome and muscle formation except in the case of the musculature of the limbs. Consequently, Engart, 1900, working with the chick, and Bardeen and Lewis, 1901, working with human embryos showed that Rabl and Patterson were right. They showed that the ventral body musculature does come from the ventral part of the myotome, but that the muscles of the limbs arise independently of the myotome.

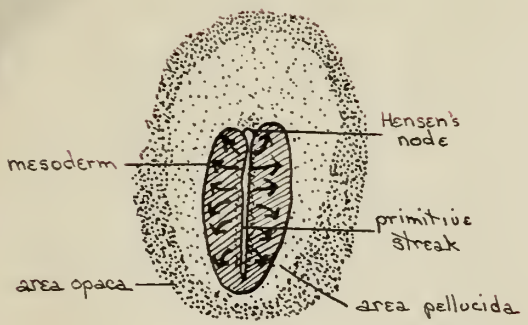
ESTABLISHMENT OF THE MESODERM TO SOMITE FORMATION

In an embryo of approximately twelve hours, the primitive streak appears as a thickening of the blastoderm marking the lines of fusion of the lips of the blastopore. The ectoderm and entoderm both merge in this region called the indifferent area of cells. The mesoderm arises from the primitive streak at the place where this merging has occurred, and grows laterad on either side of the primitive streak extending out into the space between the ectoderm and entoderm. (Fig. I) It then rapidly spreads laterad, and the anterior portion swings cephalad. Figure II shows a schematic representation of the growth of the mesoderm during the latter part of the first day. A section through the primitive streak of embryos at this stage show loosely aggregated masses of mesoderm extending to either side between ectoderm and entoderm.

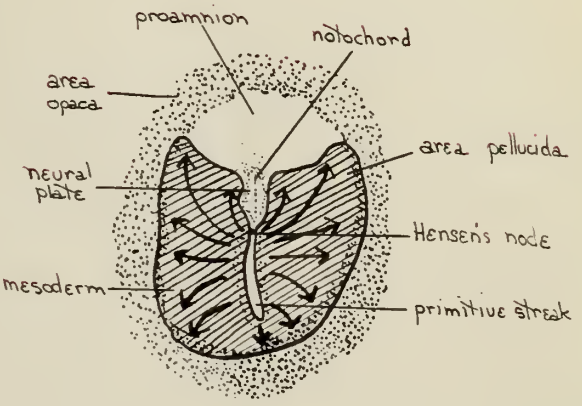
Dalton, 1935, believed that embryonic segregation occurred during primitive streak formation and that definite areas of the blastoderm had potency to form definite tissues even at that early stage. Rudnick, 1935, experimenting with grafts from embryos of this age found that considerable specificity and localization occurred dorso-ventrally during early development but that those regions lost their potency in later growth. Rawles, 1936, also working with grafts,

Figure I

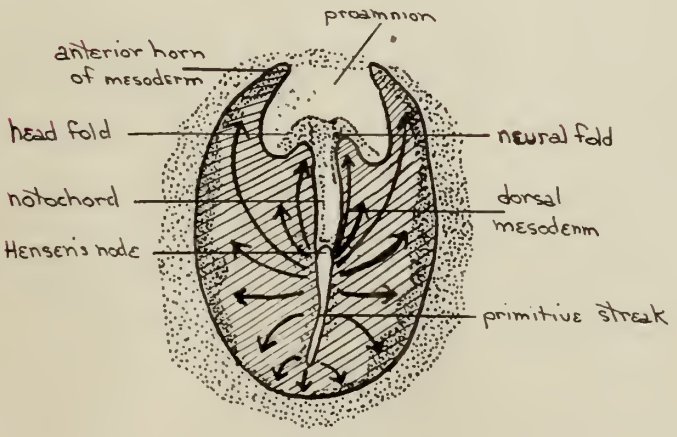
Diagram of transverse section through primitive streak showing cellular structure.
(from Patten)



Chick of about 14 hours



Chick of about 18 hours



Chick of about 22 hours

Figure II

Diagrams showing direction of growth from the primitive streak as a center. (from Patten)

found definite areas in the blastoderm of an embryo at the head-process stage had the potency to develop the various organs and tissues; the organ-forming area being fairly well restricted to the median and middle sections. Hoadley, 1936, cut the blastoderm in half and allowed it to develop in situ. He concluded that the embryo of the chick was of mosaic character.

Little mesoderm appears in the midline except posterior to the primitive streak as would be expected from its origin at the dorsal lip region of the blastopore. On either side of the midline the mesoderm is noticeably thicker than it is further laterad. In whole mounts this is quite evident by the additional opacity near the embryo. These thickened zones of mesoderm on either side of the midline are the primordia of the dorsal mesodermic plates which are called the segmental zones or vertebral plate of the mesoderm because of their later segmental character. The lateral portions become the lateral plate which by the end of the first day have split to form the somatic and splanchnic mesoderm.

A transverse fissure appears in the thickened mesoderm of the vertebral plate midway between the anterior extremity of the embryo and the primitive streak. It is a somewhat V-shaped cleft, prolonged laterally and slightly backwards, and marks the posterior boundary of the first pair of

somites which remain continuous with the mesoderm of the head region. (Each somite of this segment produces only one half as much muscle as the following somites. Williams, 1910). Shortly a second fissure develops posterior to the first marking off the second pair of somites. This process of segmentation goes on in a like manner posteriorly so that by the end of the first day approximately five somites have been formed and the primitive streak is correspondingly shortened. The first four pairs of somites later disappear and are included in the posterior part of the head.

That the first formed segment was also the most anterior one in the series has been the subject of much dispute. His, 1868, Kupffer and Benecke, 1879, Platt, 1889, and Foster and Balfour, 1910, all believed that each successive segment was formed anterior to the first. Patterson, 1907, and Hubbard, 1908, definitely proved by experimentation that somite formation progresses posteriorly. This was proved by puncturing the first somite with a hot needle, or by inserting a small glass pin into the first somite and then allowing the development to continue. The first formed somite was thus marked so that after further development it was readily seen that later somites were formed posterior to the first.

"The mesoderm of the head is derived from cells which become detached from the more definitely organized layers

of mesoderm lying posteriorly, and migrate into the cephalic region. For this reason, the cephalic mesoderm consists of loosely aggregated cells and never shows regional differentiations and the organization into definite layers which later appear in the mesoderm of the mid-body region. Aggregations of such wandering cells of mesodermal origin are called mesenchyme." Patten, 1929.

The development of the voluntary skeletal musculature begins with the segmentation of the dorsal division of the trunk mesoderm into primitive segments. The segments later lose their identity as such when the myotomes of the somite fuse to form a continuous column, a process which will be taken up in the differentiation of the primitive segments.

DIFFERENTIATION OF THE PRIMITIVE SEGMENTS

Each succeeding somite is larger at formation than the preceding somite, but due to the rapid growth, the anterior ones are larger at any given time, and, naturally, are at a more advanced stage. Thus we find that in the embryo with six somites and throughout the second day, the appearance of the second somite has changed. When it was first formed it consisted of a nearly solid mass of cells derived from the dorsal mesoderm. (Fig. III A.) The cells of the somite have a more or less radial arrangement. At this stage a cavity within the somite has been formed and the radial arrangement is more marked. At the nine somite stage the neural tube has closed above the second somite which now contains a core of irregularly arranged cells almost completely filling the cavity or myocoel. (Fig. III B.) The core appears to be an epitheloid syncytium with mitotic division occurring only near the basal layer. Sections which pass through the middle of the somite show that the core seems to arise from the lateral wall of the somite where it is continuous with the intermediate mesoderm. At this stage, Williams, 1910, says that the medial wall of the somite, just underlying the thick edge of the upper wall, is thin and indented by a groove which he calls the upper myotomic groove and which is the

first indication of the formation of the myotome. By the twelve somite stage a second, the lower myotomic groove, had appeared and the myotome is apparent as a narrow zone between the two grooves. While this is going on, other changes have occurred in the somite. The outer zone of cells on the ventro-mesial surface has lost its definitive boundaries and is merged with the core to form the sclerotome which acquires the characteristics of mesenchyme and later goes into the formation of the axial skeleton. (Fig. III C.) At the same time the dorsal part of the original outer cell zone keeps its definitive boundaries and epitheloid character forming the dermatome which later gives rise to the deeper layers of the integument. (Fig. III C & D) Thus it is the dorso-mesial portion of the outer zone of the somite that becomes differentiated into the myotome as above. In its formation it folds somewhat laterad from its original position and comes to lie ventro-mesial to the dermatome and parallel to it. (Fig. III D.) "The myotomes undergo the most extensive growth of any of the parts of the somite," (Patten, 1929), and eventually give rise to the muscles of the trunk.

During the third day of incubation the embryo has about thirty-six pairs of somites. Williams says that the later formed somites do not recapitulate the development of the earlier ones and that they are further along when

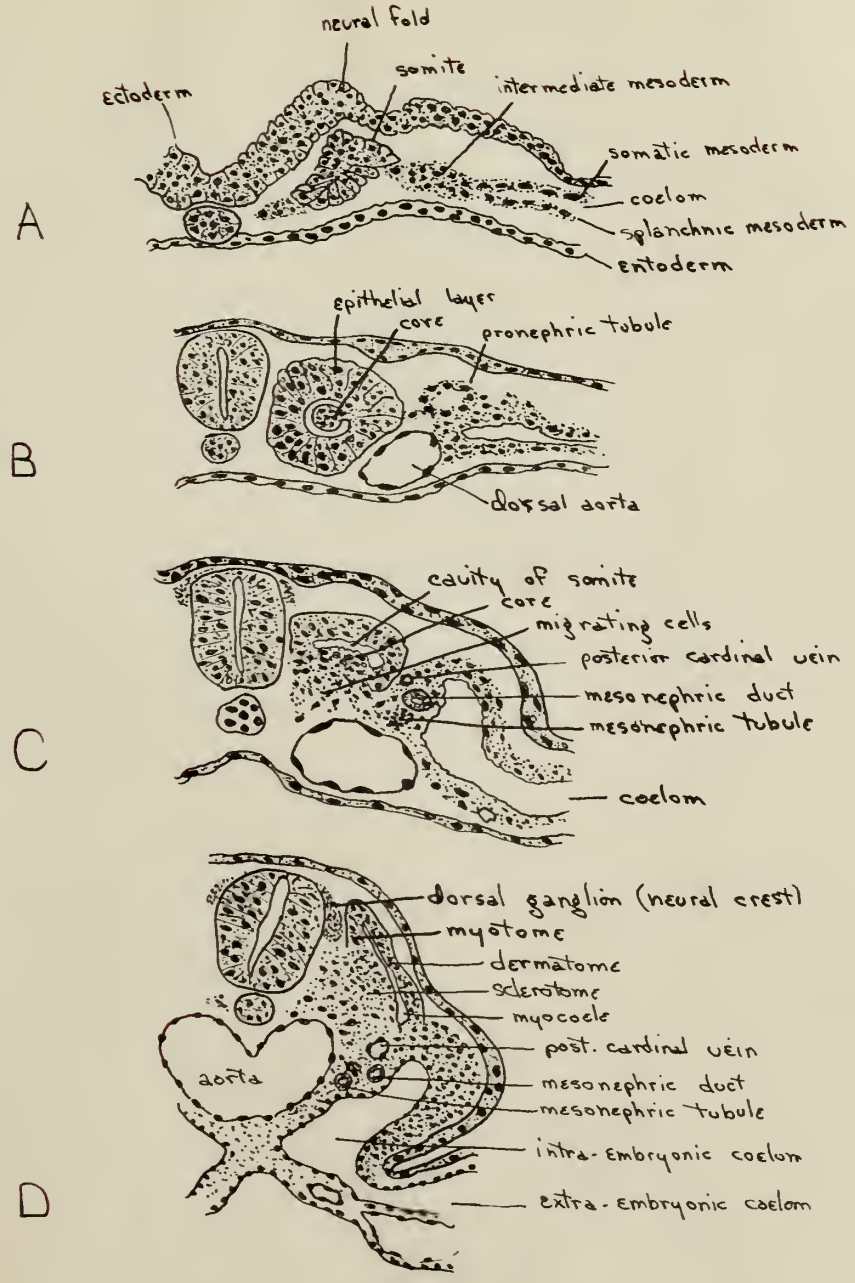


Figure III

Drawings from transverse sections to show somite differentiation

(from Patten)

formed and develop faster. But McEwin, 1923, claims that the newer, posterior somites, when first formed, are in the same condition as were those which are now anterior, and are destined to go through the same process of development. By the end of the fourth day there are forty-two pairs of somites. Ten more will be added and then disappear again similar to the first four somites. Consequently, a chick at the end of the fourth day possesses all the somites which take part in the development of the adult bird. The development of these posterior, later formed somites may be considered to be essentially the same as that given above for the second.

HISTOGENESIS OF MUSCLE

The histogenesis of muscle tissue may best be followed by separating the tissue into two groups, smooth muscle and striated muscle, and develop it accordingly. There are many steps in the process of muscle formation and they have been followed out very completely by McGill in 1907. The most noticeable change at first is that of the nuclei, and, according to Carey, 1921, "The elongation of the nuclei is the first step in myogenesis," and then later during development, "the elongation of the muscular fasiculi is in the direction of a dominant force extrinsic to the zone of myogenesis."

Smooth Muscle: Smooth muscle originates in both the ectoderm and the mesoderm. However, the bulk of it is derived from the mesoderm, either directly from the mesenchymal derivatives of the mesoderm or from the embryonal connective tissue. The mesenchyme which arises from the mesodermal germ layer is in the form of a syncytium and shows a protoplasmic continuity throughout the entire syncytial mass. The protoplasm has a fine reticular structure and the nuclei are usually round or oval. Smooth muscle does not exist at any time as separate and distinct cells and fibers. At first there is a general condensation of mesenchyme and a period of cell multiplication, during

which time the nuclei elongate in the regions which are differentiating into muscle tissue. The other mesenchymal cells, not forming muscle tissue are the anlage of the interstitial connective tissue. In later stages muscle is developed from a more developed mesenchyme or embryonic connective tissue, as for example in the musculature of the alimentary tract. The circular muscles appear long before the longitudinal muscles do. The amount of protoplasm surrounding the nuclei increases to form spindle-shaped cells but still retains its syncytial character. Mitosis is rarely seen in the developing muscle but it is abundant in the mesenchyme.

Following the process of elongation of the mesenchyme, or during the stages in embryonal connective tissue development, myofibrillae are formed in the protoplasm of the elongating cells or nuclear masses. Formation of two kinds of myofibrillae occurs, coarse and fine. The development of smooth muscle may now be divided into two periods. The coarse myofibrillae which develop first stretch out until they appear as more or less distinct longitudinal striations which are rather granular. By this time the protoplasm of the cells is largely granular instead of the fine meshed network or reticulum although the reticulum is still visible at their margins. The granular stage does not persist as resulting condensations and fusions produce

fibrillae that apparently are solid varicose and smooth. In the second period of smooth muscle formation the fine myofibrillae from embryonal connective tissue appear to arise directly as such without passing through a coarse stage, and the coarse fibrillae arise by an increase in caliber or by the fusion of several fine myofibrillae into a compact bundle. These coarse fibers differ from the coarse fibrillae of the first period in that they are larger and show no spindles. The collagenous fibrillae form throughout the mesenchyme and in the smooth muscle syncytium indicating the origin at this time of muscle from the embryonal connective tissue syncytium in which these fibrillae have already developed. The early development of the smooth muscle and connective tissue shows that the connective tissue does not invade the muscle but that it arises in situ.

After the layers of smooth muscle have been established the tissue may increase by two ways: (1) by the continuation of the transformation of mesenchyme at the surface layer or by the transformation of the interstitial cells; and (2) by multiplication by mitosis of the nuclei in previously formed muscle. This may occur particularly during the advanced stages.

Cross-striated: Cross-striated muscle arises from mesoderm, but there is a question whether the adult

multinucleated cells arise from a single cell whose nucleus divides many times or from the fusion of several cells. Bardeen, 1900, said that the first muscle cells are derived by the elongation of epithelial cells of the myotome. These then give rise to the myoblasts, round to ovoid cells without a definite form but with active mitotic division. Some of the myoblasts elongated at once to form muscle cells and others continued division. The cross-striated cells come only from the myoblasts and by elongation and differentiation. None come from the previously differentiated cells by transverse or longitudinal splitting. Godlewski, 1902, was of the opinion that the multinucleated cells of the adult cross-striated fiber arose from the fusion of several cells.

In the myotomes, muscle differentiation progresses from the anterior to the posterior region. The cells form a syncytium which may extend from one myotome to another. At first the cells are cylindrical and epithelial-like, but later they are observed to contain small round granules scattered throughout the cell. These granules become arranged in rows and migrate peripherally to form the rudimentary fibrillae. As the latter develop, the granules become closer together by increasing in number until they form a continuous thread. This shows a striking similarity to smooth muscle development. (In order to show this

fundamental similarity in a more striking manner, the work by Carey, 1921 has excellent results. He subjected the growing vesicular smooth muscle of the bladder to a stimulus comparable with that of the heart and obtained cross-striations in his tissue.) The developing fibrillae of the striated muscle increase in length and soon extend the entire length of the myotome. The granules between the fibrillae become rare as the fibrillae increase in number. The latter then become arranged more and more parallel to the cell axis and they are grouped about the nuclei. The connections of the fibrillae from myotome to myotome give the appearance of one complete mass of myotomic tissue. The fibrillae gather in columns, and often fuse with the columns in the next myotome, even extending through several myotomes. Many of them, however, end at the myosepta in club-like thickenings and often have tufts of fine fibrillae. These are the components of the primitive fibers. The simple fibrillae soon show a segmentation into two differently staining substances. The deeply staining part corresponds to the Q anisotropic band of the adult fiber, and the portion which does not take a deep stain corresponds with the I isotropic band.

What is considered the second period in the formation of striated muscle begins with physiological degeneration. According to Godlewski, 1902, in certain regions of the

developing muscle, the fibrilla columns break up and disappear. The nuclei become irregular and the direction of the long axis is altered from being parallel to the fiber to being diagonal or crosswise to it. The protoplasm becomes homogeneous and gathers into stellate masses around the nuclei. The nuclei are pale and contain small amounts of chromatin. Vacuoles may even appear in the degenerating fiber. Many of the degenerating muscle fibers disappear, and then new fibers are formed by a longitudinal splitting of the normal ones already present. The newly formed fibrillae group into columns in such a manner that similar cross-striations (Q and I bands) in the same muscle fiber lie in the same plane. At a later time the Z and M zones appear.

In the development of the myotomic muscles there is probably no direct transformation of mesenchyme cells into myoblasts as Bardeen believed. Instead, the muscles from the mesenchyme are formed but they do not last and the new muscle fibers arise from the cleavage of those present. Multiplication of the fibers ceases shortly after hatching, and then the size of the muscle is increased by growth.

MUSCLES OF THE TRUNK[1]

At approximately the sixth hour of the third day of development, the muscle plate has extended a short distance down the body wall into the somatopleure. This extension continues until by the fourth day it is beyond the root of the limb buds. The muscle plate grows into the body wall between the limbs and forms the basis of the muscles of the trunk although in the region of the limbs it retains its primitive position and has no share in the formation of the limb muscles. Thus the intrinsic muscles of the trunk are derived directly from the myotomes and they include the deep muscles of the back (those beneath the serratus posterior inferior and superior), the ventrolateral muscles of the thorax and the abdomen, and the subvertebral muscles.

The Deep Muscles of the Back: The deep muscles of the back arise from the myotomic column. Vascular mesenchyme grows into the column and divides the myotome into a dorsal region which gives rise to the spinalis and longissimus groups, and a ventrolateral region which gives rise to the iliocostalis group. The groups are separated into the various muscles by the ingrowth of mesenchyme, or vascular connective tissue, in such a manner that all superficial

[1]The bulk of the work on the trunk musculature was taken from that section by Lewis in Keibel and Mall, 1910.

traces of segmentation are lost. It is in such way that the dorsal musculature is derived from the separation of the longissimus into several parts, the rectus muscle is separated from the intercostals, and the external oblique and the external intercostal muscles are both derived from the internal intercostal mass. The development of the fascia lumbodorsalis is parallel with that of the myotome column and its derivatives. Consequently, it is important as a land-mark between the muscles of true myotomic origin and those which subsequently migrate into a more superficial region. Such muscles as the trapezius, rhomboideus, latissimus dorsi, and part of the serratus posterior migrate into this region. The growth of the dorsal muscles towards the mid-dorsal line is dependent upon the extension and formation of the vertebral arches.

The Thoraco-abdominal Muscles: The ventral margin of the myotome column grows ventrally into the body wall and gives rise to the muscles of the neck and the thoraco-abdominal walls. The extension of the myotomes lies partially between the ribs and lateral to them. At this point the myotomic extensions fuse in a continuous lateral sheet which extends beyond the tip of the ribs and ends in the rectus rudiment. It also fuses over the medial surface of the ribs to form a continuous medial sheet which is continuous with that of the rectus rudiment. During this time

the muscles are beginning to differentiate in the manner discussed above. The rectus abdominis is set off as a longitudinal splitting of the muscle column formed by the fusion of the ventral ends of the myotome processes. The obliquus abdominis externus, and (more dorsally) the serratus posterior, superior and inferior, arise from a tangential splitting of the lateral sheet. The medial sheet gives rise to the obliquus abdominis internus and the transverse abdominis muscles, while the portions of the myotome processes remaining between the ribs give rise to the intercostals externus and internus. The ventral ends of the myotome fuse to form the rectus muscle.

The Subvertebral Muscles: The subvertebral muscles of the cervical region are formed by the growth of the myotomes on to the ventral surfaces of the vertebral bodies and subsequently the myotome processes fuse into a continuous column to form the longus colli muscles and the capitus. Thus it may be seen from the origin of the muscles in the previous group and here that the cervico-thoraco portion of the myotomic column gives rise, by longitudinal and tangential splitting and coincident migration, to the long muscles of the back and neck.

The perineal musculature arises from a primitive sphincter cloacal muscle which is probably derived from the ventral portions of the third and fourth coccygeal myotomes.

MUSCLES OF THE APPENDAGES

The anterior and posterior appendage buds appear as rounded swellings on the body wall of an embryo three days old. The anterior bud appears opposite somites seventeen to nineteen inclusive, and the posterior bud appears opposite somites twenty-six to thirty-two inclusive. The buds are filled with densely packed mesoblastic cells without any distinct arrangement. During the fourth day the swellings have increased considerably in size but in no other way. By the fifth day they have the appearance, somewhat, of limbs, and begin to show evidence of joints. Development increases until by the seventh day the limbs are distinctly jointed. At the close of the eighth day, Romer, 1927, says that the musculature is a miniature replica of the adult.

The principal and probably the only source of the limb musculature is from the undifferentiated, diffuse mesenchyme which forms the core of the limb bud. It has been mentioned before that there has been much disagreement over the origin of the limb muscles. Some writers, Kaestner, 1890; Kollman, 1891; Lillie, 1908; and Foster and Balfour, 1910, believed that the muscles are derived from the myotomes. Patten, 1929, also gives the impression that the limb muscle are of myotomic origin. Ingalls, 1907, ob-

served cells to enter the limb bud from the ventral margins of the myotomes and then lose their myotomic connections and become mingled with the mesenchyme. Fischel, 1895, and Jordan and Kindred, 1926, also believed that the mesenchyme of the limb buds received additional cells from the myotome. Other writers; Patterson, 1888; Engart, 1890; and Bardeen and Lewis, 1901, claimed that the myotomes played no part in the formation of the limb musculature. In 1898, Miss Byrnes believed that the limbs were wholly somatopleuric in origin; that the myotome processes took no part in the formation of the limbs but only gave rise to the musculature of the body wall; and that there was no proliferation of cells from the myotomes in the limb region. To substantiate her claim, Miss Byrnes destroyed the myotomes in the leg region of amphibia and allowed the embryo to continue to develop. The leg on the injured side showed muscle development almost as advanced as that on the uninjured side. From this experiment she concluded that, "the power to develop striated muscle is not restricted to the myotomes but that the mesenchyme-like cells of the somatopleure can and do give rise to the voluntary muscles in the limbs." Another experimenter is Spurling, 1923, who attempted to find the effect obtained by the extirpation of the posterior limb bud of the chick, suggesting that if the myotomes affected the developing limb regeneration

should take place. Former work done by Byrnes, Braus, Harrison and others showed the power of regeneration in amphibia. The only other work done on the chick was by Lillie who found no evidence of wing regeneration. Spurling removed the newly-formed limb buds of chicks that had been incubated sixty-five hours and obtained not the slightest trace of regeneration. In my own work with chick embryos of seventy-two hours in which somites seventeen to thirty-two inclusive were destroyed with a hot needle, muscle development similar to that of the normal leg was observed in the embryos which survived the operation for at least three days. In two embryos which lived twelve and thirteen days after the operation, the only difference between the limb on the injured side and that of the normal limb was that of size. These results suggest that myotomes seventeen to thirty-two do not play any part in the muscle formation of the chick appendages. The difference in size might possibly be explained as a retardation due to shock. "The limbs buds both phylogenetically and ontogenetically are of later origin than the branchial arches, and thus there is even less reason for assuming that the limb muscles are derived from the myotomes than that the branchial muscles have a similar origin. As the limbs are of later origin than the trunk musculature, it is but natural to expect the limb musculature to arise independently."

Lewis, 1910. The forelimb differentiates first, and in both appendages differentiation starts in the proximal portion, thus the feet of the chick are the very last to be formed.

Despite the fact that a distinct myotomic source of mesoderm has not been completely demonstrated in the limb buds, the nerve relationship gives the muscle a secondary segmental character. The primordial premuscle masses differentiate into preaxial and postaxial masses with reference to the longitudinal axis of the limb. The nerve fibers go in pairs, one branch to the preaxial, the other to the postaxial. Then by longitudinal and tangential splitting, and by migration, the primordial masses give rise to the definitive musculature of the limbs. The long axis of the primitive limb buds are at first parallel with the long axis of the body but there is an early shift to right angles. (Fig. IV) The pre- and postaxial surfaces of both limbs are in the same relative position. This is followed by a rotation through a ninety degree arc and the limbs are again parallel with the body. However, the rotation occurs in opposite directions so that the definitive muscles on the outer surface of the upper limb (preaxial in origin) are homologous with those on the inner surface of the lower limb. (Fig. IV B.)

Muscles of the Shoulder Girdle: The trapezius and

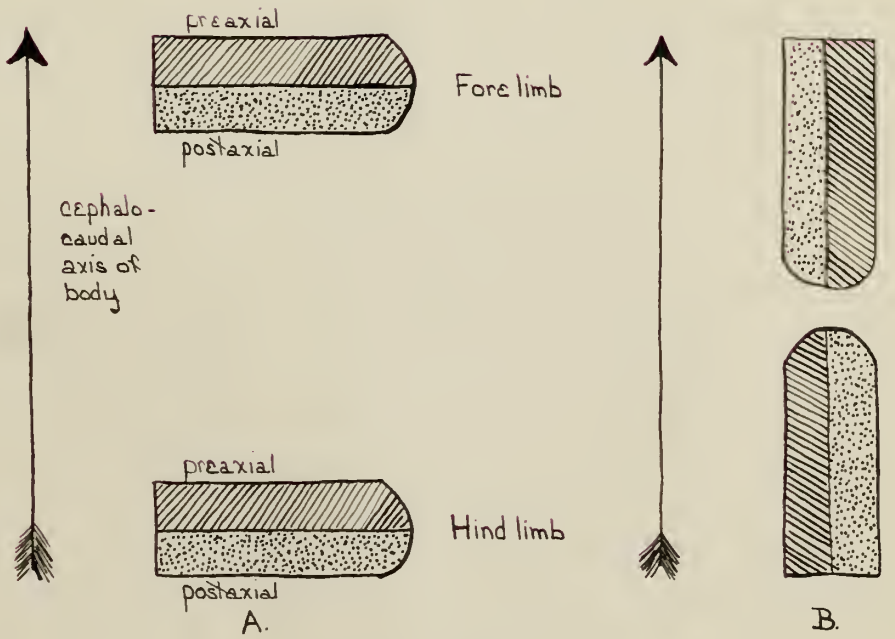


Figure IV

Diagram showing the shift in position of the limbs in relation to the body axis.
(From Jordan and Kindred)

sternocleidomastoideus from their positions and nerve supply are regarded as a caudal member of the branchial arch series. At first they are merely a mass of closely packed cells containing the nervus accessorius. This mass is extended almost to the point where the accessorius leaves the vagus and gradually extends caudally toward the forelimb and splits into its two parts. The sternocleidomastoideus later splits to form the sternomastoid and the cleidomastoid. The rhomboideus major and minor, the elevator scapulae, and the serratus anterior all arise in the lower cervical region. The subclavius muscle develops in situ from the mesenchyme of the cervical region before the other muscles migrate caudally. It is carried along by the first rib. The omohyoideus splits off from the infrahyoid muscle mass and becomes attached to the scapula. The latissimus dorsi and the teres major muscles are closely associated in their origin from the premuscle sheath of the wing. The teres major develops in situ, but the latissimus dorsi spreads caudally over the side of the thorax. The pectoralis major and minor muscles are derived from a premuscle mass in the lower cervical region on the median side of the forelimb bud. As it differentiates it flattens out and extends caudoventrally into the region of the distal ends of the upper ribs. The major portion becomes attached to the humerus, and the minor portion becomes attached to

the coracoid process and the second, third and fourth ribs. The intrinsic muscles of the wing develop in situ and have approximately the same position as in the adult form. The tendons also develop in situ at the same time so that the muscles from the first are in connection with the skeletal structures. Of the remaining muscles, the deltoideus, teres minor, supra and infraspinatus arise from a common premuscle mass that is continuous with the pectoral muscle mass. The subscapularis is more or less isolated from the others from the first. It occupies a small portion of the median surface of the scapula and later extends until it covers the entire medial surface. The triceps muscles arise along the posterior and lateral surfaces of the humerus and extend from the scapula to the ulna.

Patterson, 1888, stated that in the limbs of birds, the muscles are produced by further differentiation of the dorsal and ventral strata which appear from the mesoblast cells in the original limb bud. These cells form a central cartilaginous bar, above and below which are developed the muscle layers. Carey, 1921, claimed that the formation of muscle tissue is a function of its position. It is formed in situ and then is dependent upon an optimum tension which is elicited by a dominant zone of accelerating growth. This forces, by traction, a zone retarded in growth. Thus he claimed that the two zones exhibiting a tensional interaction

give rise to the differential growth of the skeletal musculature.

Muscles of the Thigh: The earliest stage which shows differentiation of the myoblasts into separate components occurs during the sixth day of incubation. The preliminary formation of the limb musculature into a dorsal and ventral mass has been mentioned previously but will be reviewed again in a more detailed account of the development of the thigh musculature. Romer, 1927, has been the chief contributor to the following account.

The dorsal mass of muscle tissue takes its origin proximally on the dorsal and anterior surfaces of the femur at about the future site of the head of the adult femur. The mass then passes over these surfaces to the knee region, and then travels downward over the extensor surface of the tibia and the fibula. It terminates in the region of the future metatarsals. The ventral mass of muscle tissue takes its origin slightly more proximally than dorsally, and passes down the ventral and posterior surfaces of the femur to the knee. At the knee it curves sharply and runs down the flexor aspect of the lower leg. The ventral mass also terminates in the metatarsal region. (Figs. V A. & VI A.)

Because many of the important nerves are encountered throughout the development of the thigh musculature and are very easily followed they are included in this account

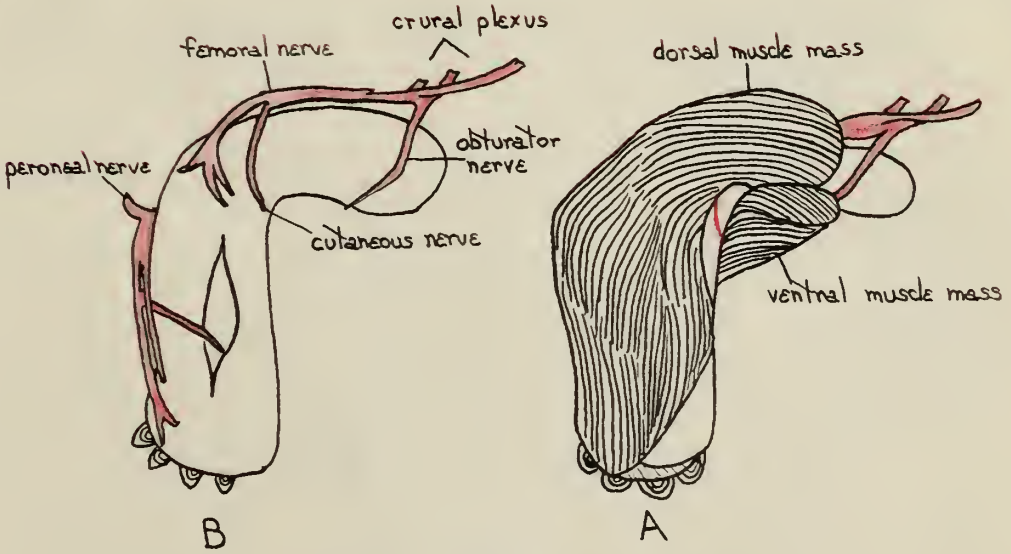


Figure V

Right Pelvic Limb of 6 Day Chick - External View
(from Romer)

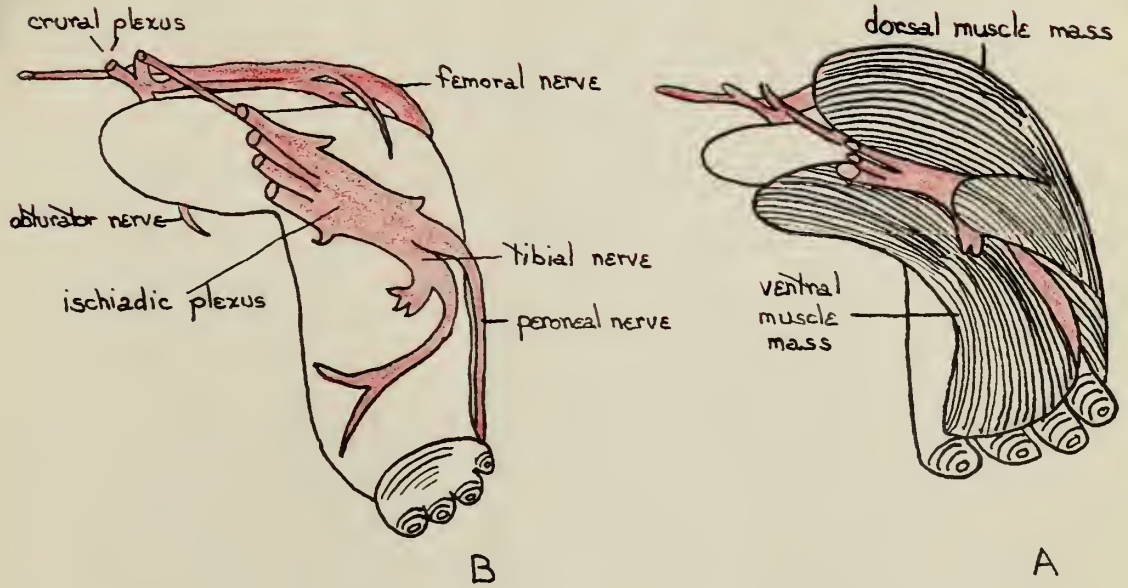


Figure VI

Right Pelvic Limb of 6 Day Chick – Internal View

(from Romer)

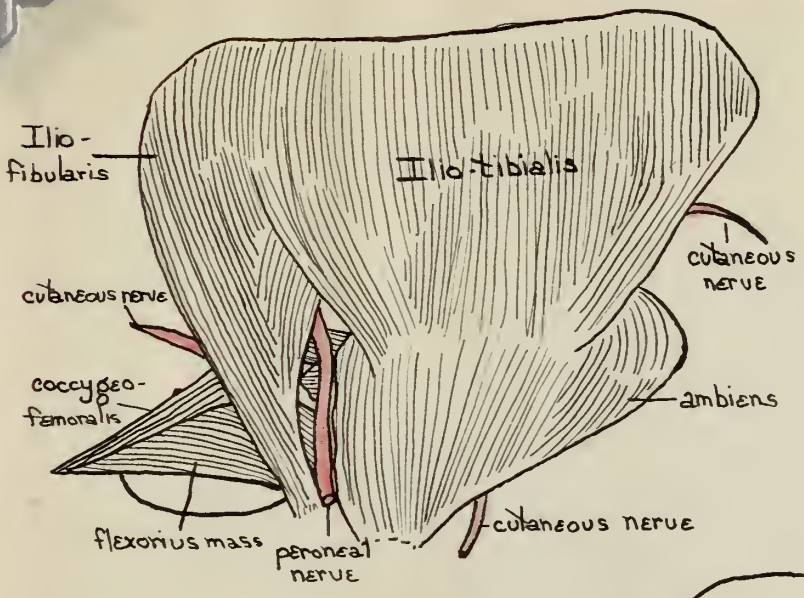
instead of with that of the other nerves. The crural plexus is formed from the two most anterior roots of the lumbo-sacral plexus and from a branch of the next posterior nerve, (the nervus furcalis). As the two roots of the plexus approach one another above the rudiment of the pelvic girdle, a branch is formed which passes around the anterior or external side of the fused femur and pelvis and ends ventrally in a portion of the ventral muscle mass. The nerve so formed is the obturator nerve. The femoral nerve is formed just beyond the point at which the obturator is given off, and then continues through the dorsal mass to the knee region where it terminates. A cutaneous nerve emerges on the anterior side of the leg near the knee. The furcalis nerve and the four roots posterior to it give rise to the ischiadic plexus. (Figs. V B. & VI B.)

A longitudinal division of the dorsal and ventral muscle masses takes place in the knee region separating entirely the musculature of the lower leg from that of the thigh except for connections through the flexor system. The posterior margin of the superficial portion of the dorsal mass is now innervated by a small branch from the ischiadic plexus but the main innervation of the mass is still from the crural plexus. The dorsal mass is quite rapid in its development but the ventral mass proceeds more slowly. This mass is innervated almost entirely by the branches of the

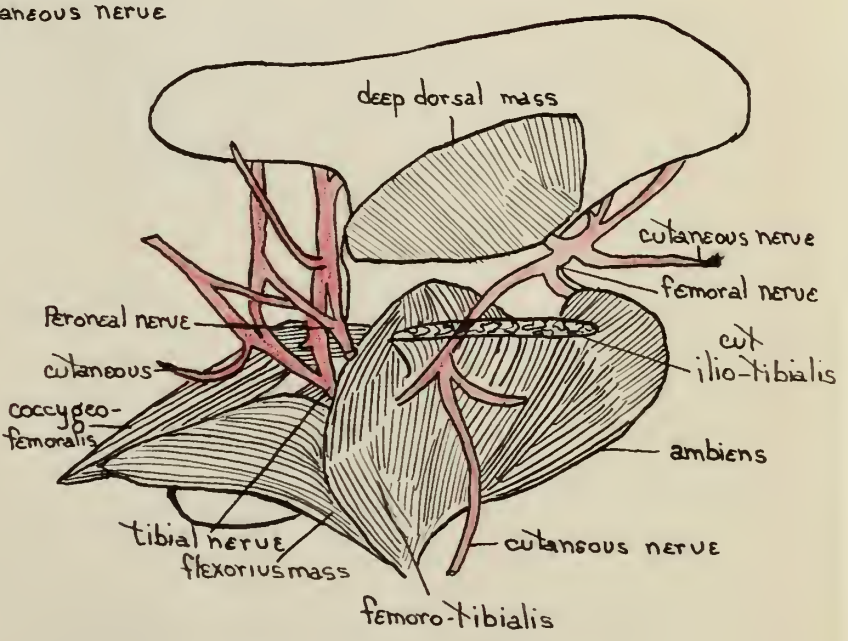
ischiadic plexus, but that portion of the mass which will give rise to the pubo-ischio-femoralis and obturator internus muscles are innervated by the obturator nerve. (Fig. VII B. C.)

By the seventh day of incubation the muscle has differentiated to a considerable degree and is beginning to assume the adult appearances. It continues to increase in size and the distinctions become more noticeable until by the end of the eighth day it is "now a miniature replica of the adult except for some minor changes which are to take place." (Romer, 1927)

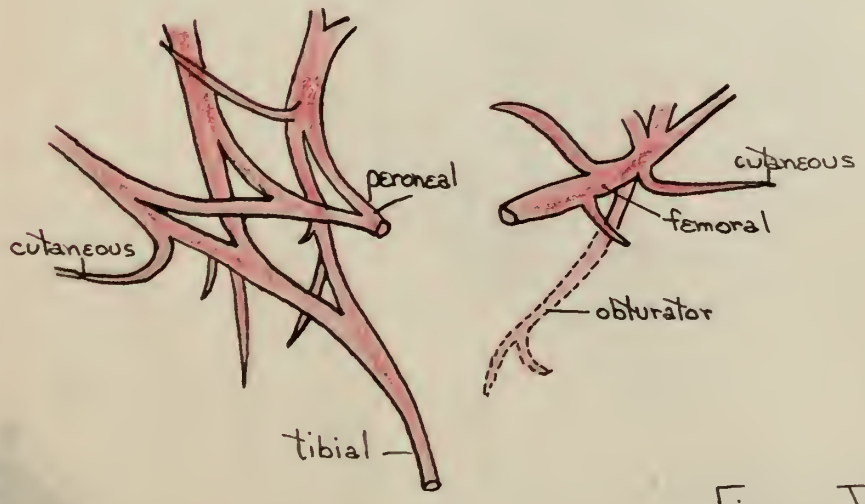
For the separation of the actual muscles it will be clearer to follow the account with figures V, VI, VII, and VIII. The deep dorsal muscle mass gives rise to a superficial dorsal mass and a deep dorsal mass. The muscles of the triceps femoris and the ilio-fibularis are derived from the superficial dorsal mass. In a later stage the ilio-tibialis, the ambiens, and the three heads of the femoro-tibialis originate from the triceps femoris. At a very advanced stage the ilio-tibialis gives rise to the sartorius muscle and the two heads of the ilio-tibialis proper. From the deep dorsal mass of the thigh musculature emerge the ilio-femoralis externus, and internus, and the ilio-trochanterici. The latter later splits up into its adult form of three heads. The derivatives of the ventral muscle



A. Late 6 Day Chick
Thigh Region
Lateral View



B. Late 6 Day Chick
Thigh Region, Lat. View
Ilio-tibialis and Ilio-fibularis removed



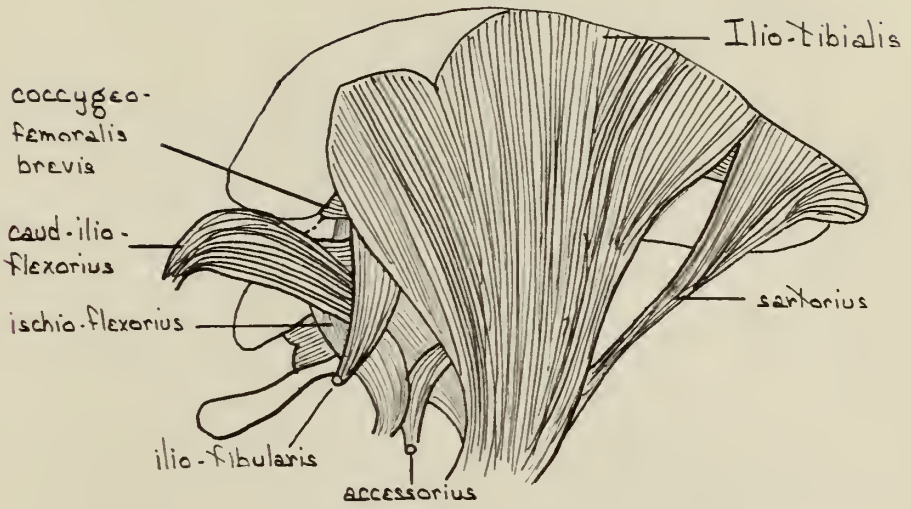
C. Late 6 Day Chick
showing nerve tracts

Figure VII (from Romer)

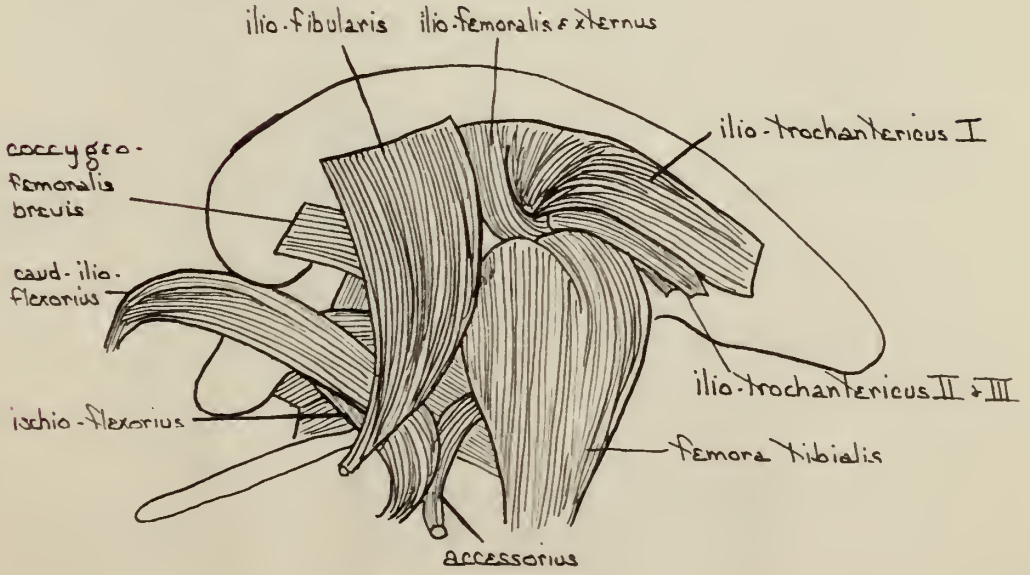
Figure VIII

8 Day Chick - Lateral Superficial View

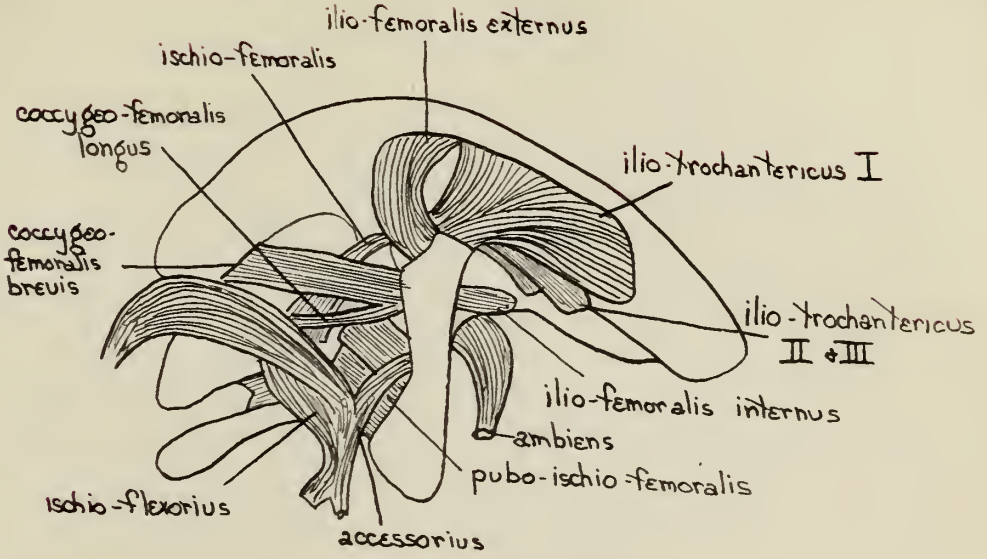
(from Romer)



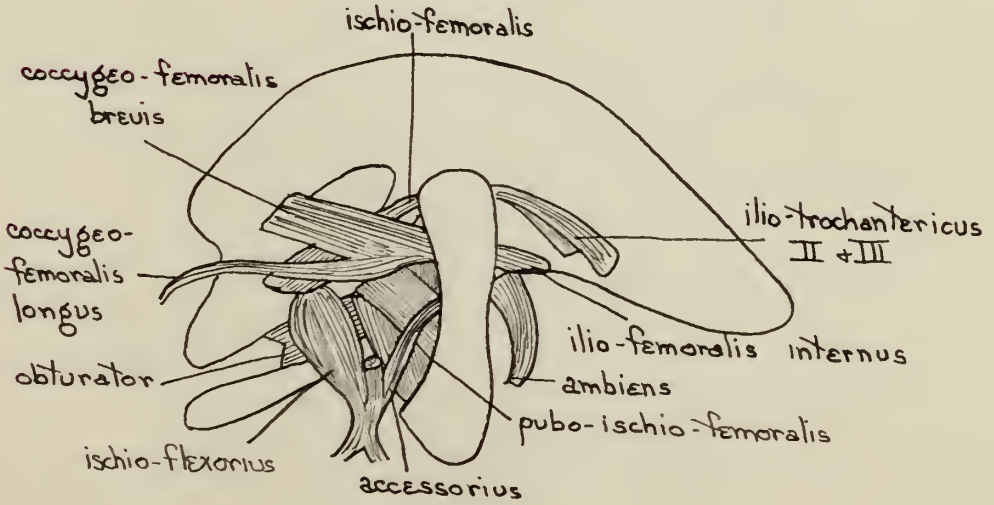
A.



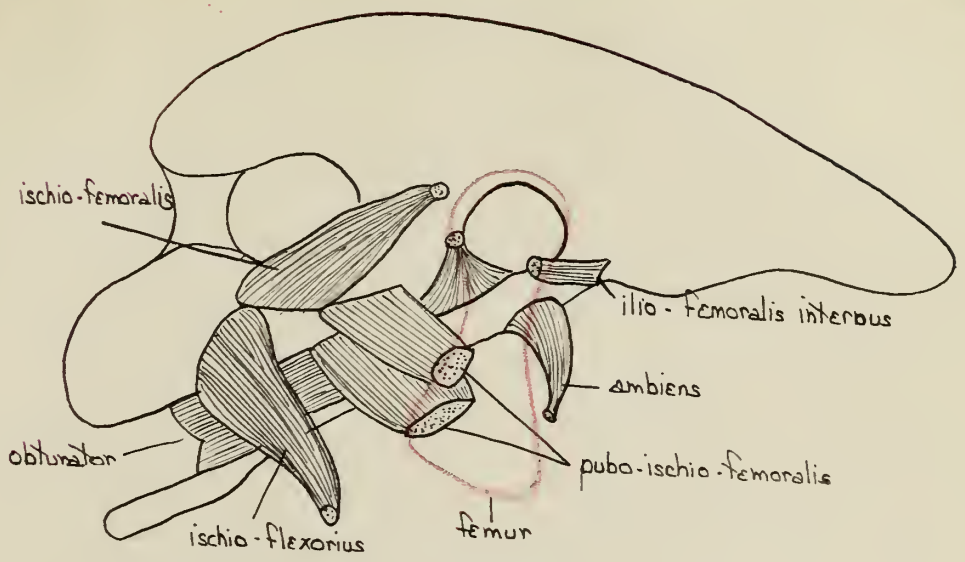
B. Ilio-tibialis and Sartorius removed.



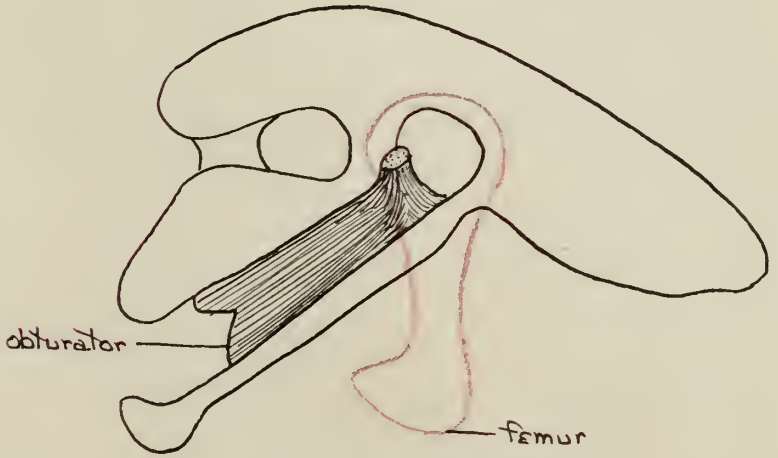
C. Ilio-fibularis and Femoro-tibialis removed.



D. Ilio-femoralis externus, Ilio-trochantericus I and Caud-ilio-flexorius removed.



E. Ilio-trochantericus II, III, Coccygeo-femoralis and Accessorius removed.

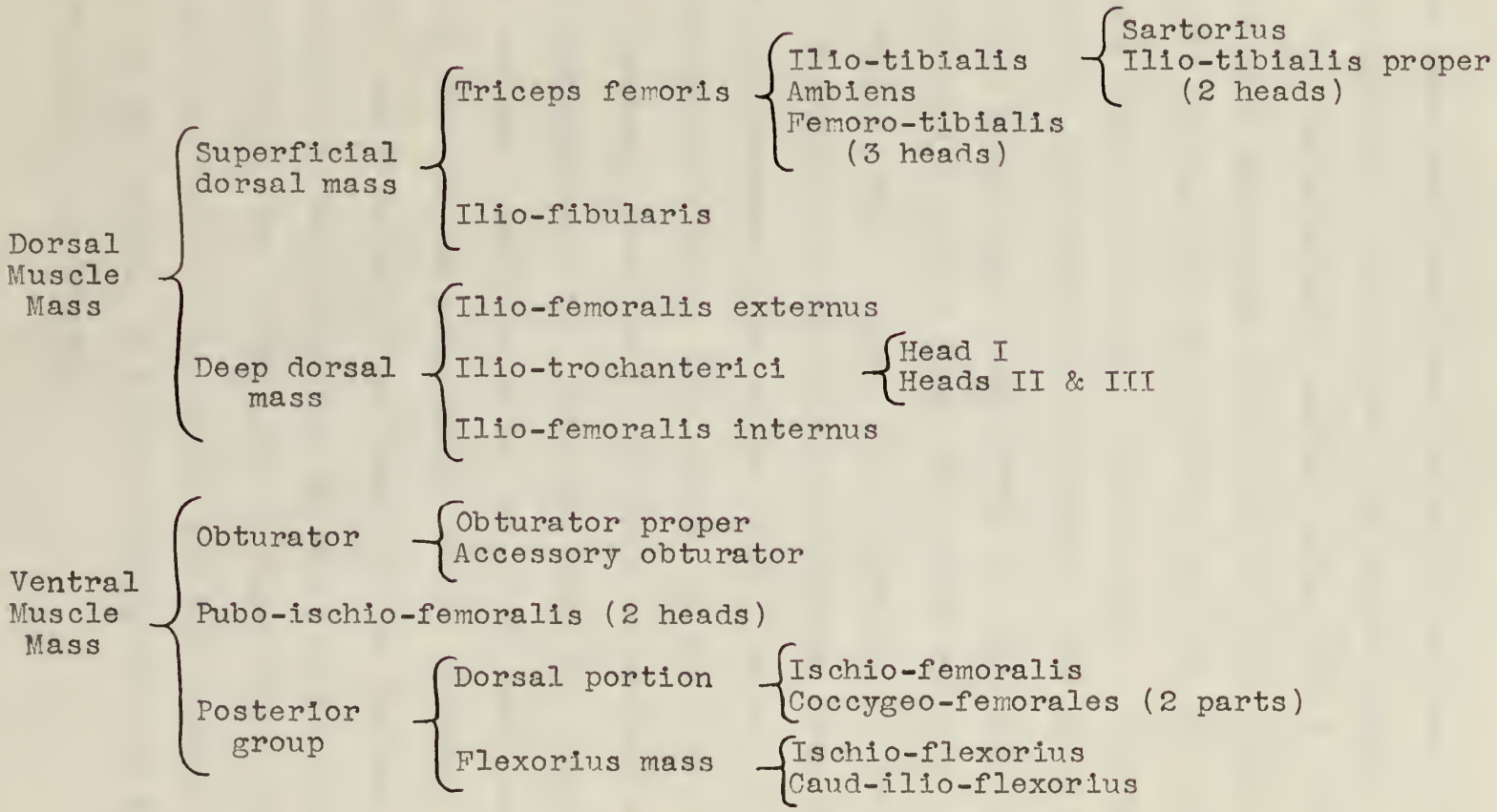


F. Ischio-femoralis, Ilio-femoralis internus, Ambiens, Pubo-ischio-femoralis and Ischio-flexorius removed.

mass, in a sense, are not quite so complicated. It gives rise to three groups of muscles; the obturator, pubo-ischio-femoralis, and the posterior groups. In the first group the obturator muscle mass gives eventual rise to the obturator proper, and also to the accessory obturator. The pubo-ischio-femoralis of the second group later splits into the two heads of the adult form. The posterior group of the ventral muscle mass shortly redivides into a dorsal portion and a flexorius mass. The ischio-femoralis and the coccygeo-femoralis muscles are derived from the dorsal portion, and in later stages the coccygeo-femoralis separates into the coccygeal and the femoralis muscles. The flexorious mass gives rise to but two groups of muscles, the ischio-flexorius and the caud-ilio-flexorius muscles. Table I shows the development of the definitive muscle from the original dorsal and ventral muscle masses.

TABLE I

Mode of differentiation of the thigh musculature of the chick.



MUSCLES OF THE HEAD AND NECK

It has been mentioned that the cervico-thoracic portion of the myotomic column gives rise to the muscles of the neck. The ventrolateral muscles arise from a closely packed premuscle mass which gives rise to three goups: (1) the infra hyoids, (2) the scaleri, and (3) the levator scapulae and serratus anterior. Hunter, 1935, followed the hypoglossal musculature development of the chick and found that the hypoglossal down-growth is initiated as a ventral extension from the third and fourth myotome at about the second somite stage. The descending myotomic processes subsequently form a common condensed area that is ventral to the myotomes from two to seven. He calls this area the submyotomic tract, from which, at the level of the fourth myotome, develops a cord-like concentration of cells, the hypoglossal cord. Myotomes one, two, six and seven are indirect contributors to the hypoglossal musculature, giving rise to the submyotomic tract. Myotomes three, four, and five are direct contributors because they produce definite ventral processes at the site of attachment of the hypoglossal cord. This cord is later associated with the anterior wall of the aortic sac just ventral to the anlage of the thyroid as well as giving rise to the chief hypoglossal musculature.

The muscles of the head originate from a loose aggregate of mesenchyme cells which are derived from the cephalic portion of the mesoderm when it is first formed, and also from the first four original somites which have been incorporated with the head region as the four head myotomes from the mandibular, the hyoid, and the first and second branchial segments.

With the exception of the lens muscles which are derived from the ectoderm, many of the muscles of the eye come from the pre-mandibular segment. (Fig. IX.) The pre-muscle mass of mesoderm in the region of the eye orbits may be homologous with the head myotomes of the elasmobranchs. Such a homology is indicated by the innervation of the extrinsic eye muscles by the somatic motor cerebral nerves. According to Minot, 1911, "the anlage of the muscles of the eye, (these are, by hypothesis, homologous with the cells and the walls of the head cavities of the lower vertebrates which produce the muscles of the eye) may be seen in the 10 mm. pig embryo between the anterior cardinal vein and the carotid artery as a group of embryonic cells quite distinct from the surrounding mesenchyme." The superior oblique muscle comes from the upper end of the mandibular myotome which also gives rise to the depressor palpebrae inferioris and the orbito-quadratus muscles. The lower portion of this same myotome goes through three stages in

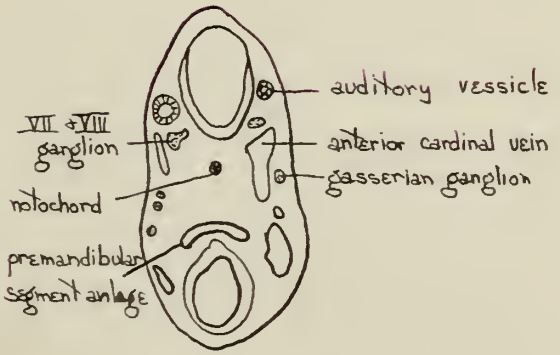


Figure IX

Transverse Section thru Chick Embryo Showing Fully Formed Anlage of the Premandibular Segment. (from Edgeworth)

the development. The muscles which eventually develop from the premuscle masses of the branchial segments differ from all other skeletal musculature in that they are innervated by visceral motor nerves. During the so-called first stage, a single muscle mass is formed which extends from the pterygoid process to the lower jaw. In the second stage of development the single mass divides into two portions, an inner or pterygoid mass, and the outer or temporal mass. What is considered the third stage is the division of these newly formed nerves into their respective definitive muscles. The inner mass gives rise to the external pterygoid and to the internal pterygoid muscles. The quadrato-maxillaries and the temporal muscles are the eventual derivatives of the outer mass.

The myotome of the hyoid segment consists at first of a single muscle mass, but the abducens nerve which comes in at the upper end of the mass passes forward and internally through the mass until it reaches the Gasserian ganglion. The path of this nerve divides the muscle mass into two parts, the anterior and posterior muscle masses of the hyoid myotome. The anterior mass is the anlage of the pyramidalis and quadratus-nictitantes muscles, and the posterior portion is the anlage of the external rectus. At a later period the depressor mandibulae muscle originates from the hyoid myotome. (Fig. X.)

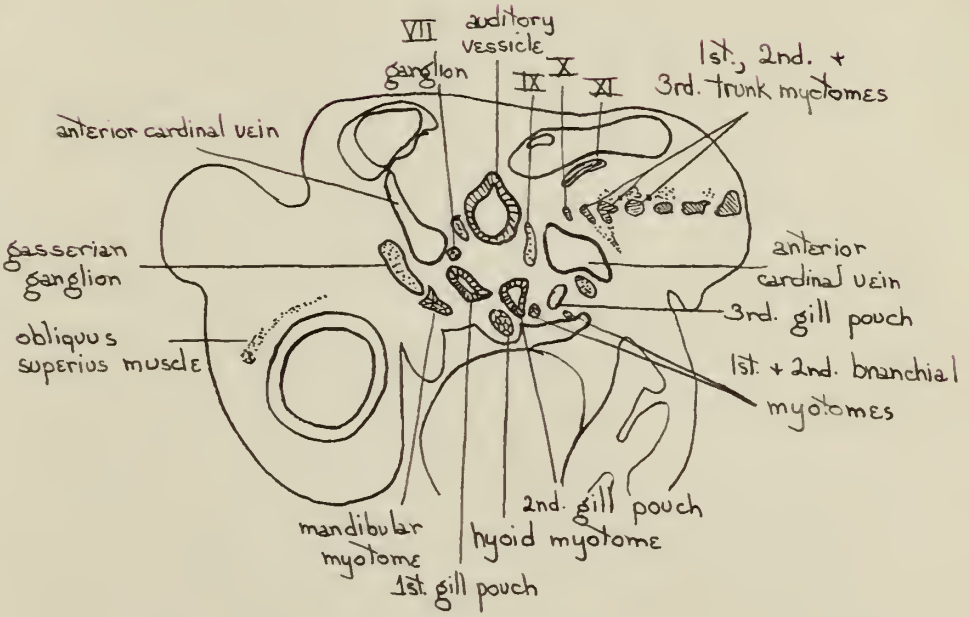


Figure X

Sagittal Section of Chick Embryo - 4 Gill Pouch Stage

It shows (partially) formation of ventral longitudinal muscles which originate from first (½) myotome and 4 succeeding ones. Owing to spiral twist of embryo no single section shows all 5 down growths.

(from Edgeworth)

The myotome of the first branchial segment is also separated into an upper and lower muscle mass. In this case, however, the upper part seems to disappear while the lower mass of muscle tissue gives rise to the branchiomaxillaris and its derivatives which extends from the first branchial bar to the lower jaw. The myotome of the second branchial segment disappears entirely leaving no trace. Early in the development of the coelom, the most cephalic portion in the region of the mandibular and hyoid segments is obliterated. The mylohyoid muscles are formed from the walls of the obliterated portion of the ventral cephalic coelom. Secondary changes occur in the mylohyoid musculature which result in the formation of the anterior constrictor colli, the serpihyoid and the stylo-hyoid muscles.

The ventral longitudinal muscles of the head and neck are derived from the first five trunk myotomes. Each of these myotomes sends out processes in the form of ventral downgrowths which curve as they progress downward. The ventral ends of these processes fuse, and then separate from the rest of the myotomes. At this stage in development the fused ventral portions appear as an elongated mass of cells in the ventral region of the branchial segments in a position just dorsal to the cephalic portion of the coelom. This newly developed muscle mass then extends both forwards and backwards, forming the anlage of the ventral longitudinal

muscles of the head and neck. (Fig. X.)

The Laryngeal and Syringeal Muscles: The anlage of the laryngeal muscles develops in the splanchnic mesoblast of the second branchial segment during the sixth day. Fig. XI shows the appearance of this region in the middle of the sixth day. By the seventh day of incubation each lateral mass of the laryngeal anlage has divided into the apertor and sphincter laryngis muscles. The syringeal musculature is derived from mesoblast cells which have spread down along the trachea. The anlage of these muscles is not visible until the eighth day.

The Lingual Muscles: The anlage of the lingual muscles is homologous with the hyo-glossus musculature of the reptiles. At first it extends from the first branchial bar to the first basi-branchial. Subsequently it grows forward and divides into the ceratoglossus, and hyoglossus, rectus and obliquus muscles. The anlage of the lingual muscles begins to form before the geniohyoid muscles are quite separated from the sternohyoid muscles. Fig. XII is a sagittal section of the head of a chick at the end of the seventh day showing the musculature developed by that time.

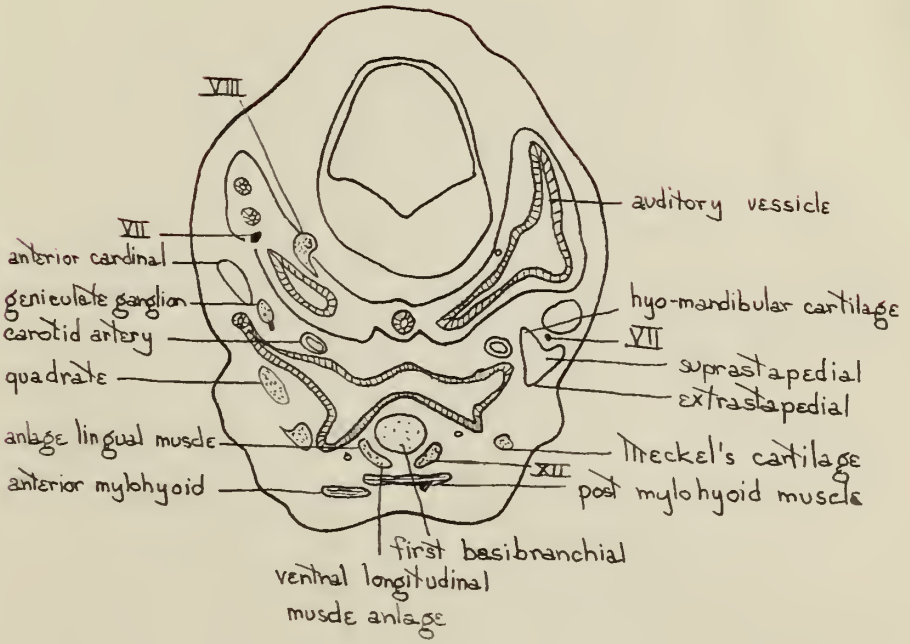


Figure XI

Transverse Section Chick - Middle of 6 Day

(from Edgeworth)

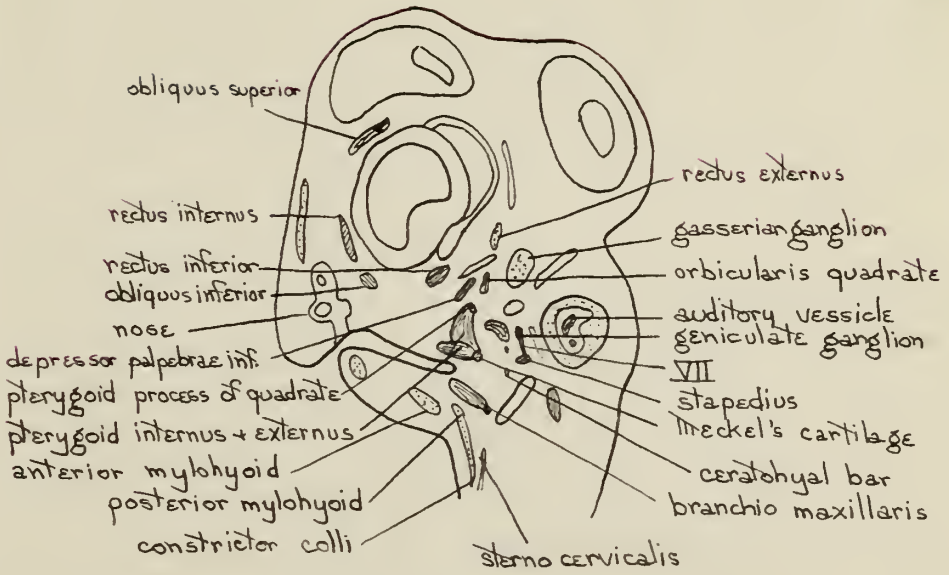


Figure XII

Sagittal Section Chick at End of 7 Days

(from Edgeworth)

INNERVATION

The treatment of the nerves and their innervation is taken separately from the preceding discussion on muscles because the available evidence seems to indicate that nerve and muscle tissue develop independently of each other, in spite of their close proximity during their development and differentiation.

Evidence from experimental embryology seems to show, contrary to former belief, that early muscle differentiation is independent of any nervous stimulation. Harrison, 1904, Hooker, 1911, and Hamburger, 1923, showed that the nerves have no effect on the muscle development in Amphibia. Since their work was done on cold blooded animals, other experiments were carried out with warm blooded animals in order to ascertain similar or dissimilar results. Weber, 1851, worked with both pig and calf embryos and said that the developing muscle is dependent upon its corresponding nerves. Bardeen, 1900, said that the appearance of the myotomes of the pig is at an entirely different time from the formation of the spinal nerves. Herbst, 1901, claimed that the nerves exert a definitive formative stimulus in the genesis of muscle. Burrows, 1911, transplanted sixty-two hour embryonic tissue consisting of three myotomes and a portion of the spinal cord. After growth had continued

for four days he found that the nerves grew out from the cord in two groups, each passing through different intersegmental spaces between the myotomes but that the myotomes appeared only as chains of slowly-growing striated cells. Anders, 1921, disagrees with this statement by saying that originally muscle tissue differentiates without nerve stimulus, but that a secondary degeneration occurs so that the muscle fibers are represented by fat lamellae. Murray, 1926, said that a bud of a three day chick can complete its development into a limb without any further influence. Strangeways and Fell, also in 1926, grew limbs excluding the nerve cells. Cartilage, bone and epidermis developed, but they found no skeletal muscle. This differentiation, however, was so atypical that they did not consider that the absence of nerve tissue as a special factor preventing muscle differentiation. Hunt, 1932, experimented with chick limb bud grafts and found that skeletal muscle will differentiate in the absence of nerves although the amount of muscle tissue is usually subnormal or degenerating. According to Carey's theory, 1921, the muscle may have degenerated because it was not attached at both ends. Hunt disagrees because when normal muscle is deprived of its nerve stimulus it atrophies and degenerates. She concluded from her experiments that the muscle of the chick limb is capable of initial independent differentiation, but the

differentiation is not always maintained independently. Some external factor seems to be necessary by the tenth day. Because the non-innervated grafts showed fatty degeneration she inferred that innervation is an important factor in the maintainance of normal muscle tissue.

A bit of further evidence for the independent development of nerve and muscle fibers is shown in the origin of muscular response. Windle and Orr, 1934, found that the first responses to mechanical stimulation occurs in the six-and-one-half to seven day chick as local reflexes which are lateral wing movements, but the contractions are not maintained. At this same time, however, there is spontaneous bilateral trunk movement present. In 1935, Windle and others found that the first muscular response was an outward and forward or backward motion of the forelimb bud in eleven millimeter rat embryos. Generally, they observed, the physiological muscular development proceeds in a very general way, caudad and rostrad from the shoulder region, and distal and ventrad from the dorsal part of the trunk. They concluded that the somatic musculature becomes functional not all at once, but progressively after an expanding pattern, independent of the muscle formation.

The Development of the Nerves and Their Innervation of the Muscles: The peripheral nervous system is comprised of the nerves and ganglia outside the central nervous system,

and thus includes the cerbrospinal nerves and the sympathetic nervous system. The afferent fibers carry sensory impulses to the central nervous system and develop from the neuroblasts of the neural crest. The efferent fibers carry the motor impulses away from the central nervous system and originate in the basal plate of the neural tube. The afferent and efferent fibers may be classified as in the following table from Jordan and Kindred:

A. Efferent Fibers

(a) Somatic terminating in skeletal muscle (Fig. XIII).

(b) Visceral

1. General; terminating in the sympathetic ganglia from which fibers extend to the smooth muscle of the viscera, glands and vasa, and to the cardiac musculature. (Fig. XIII)
2. Special; terminating in striped muscle derived from the mesenchyme of the branchial arches.

B. Afferent Fibers

(a) Somatic

1. General; terminating in the sense organs of the integument. (Fig. XIII)
2. Special; terminating in the sensory epithelia of the eye and ear.

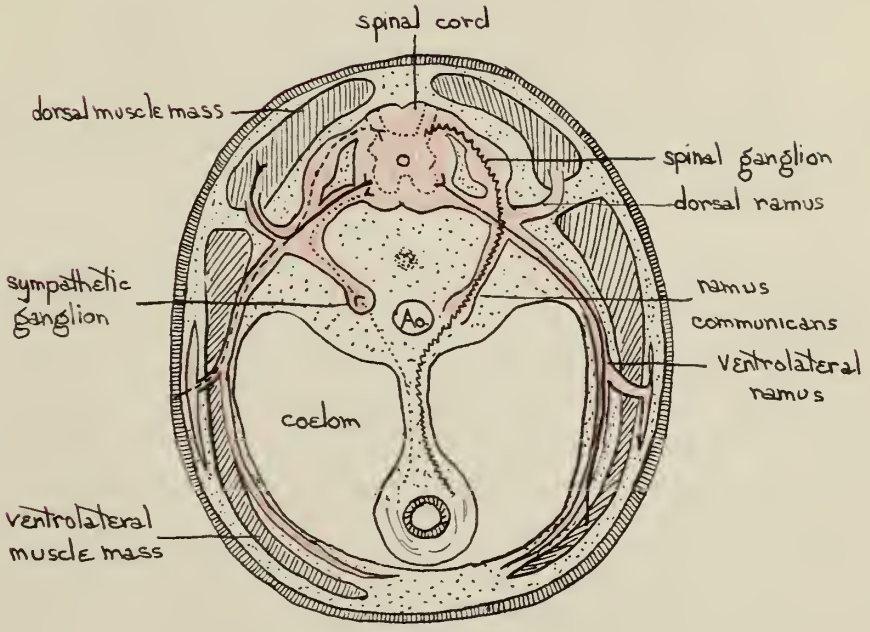


Figure XIII

Diagram Showing the Functional Parts of the Peripheral Nervous System
(from Jordan + Kindred)

solid lines – general somatic efferent fibers
dotted lines – general visceral efferent fibers
dash lines – somatic afferent fibers
jagged lines – visceral afferent fibers

(b) Visceral

1. Sympathetic; terminating in the sense organs of the viscera. (Fig. XIII)
2. Special; terminating in the olfactory epithelium and taste buds.

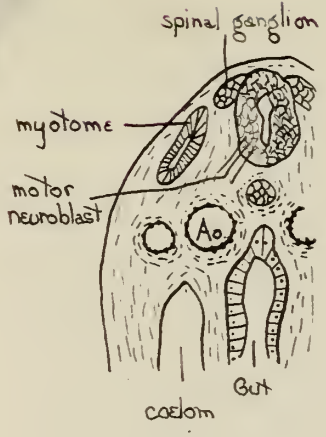
Development of the Spinal Nerves: The paired spinal nerves grow out at right angles to the spinal cord in each body segment. In the chick it has been observed (Bok, 1915) that this outgrowth normally occurs as the axones of the "activating bundle" reach their level. In amblystoma, (Yamane, 1930) it has been found that the isolation of a portion of the spinal cord from the medulla, by grafting it into the side of the body, with consequent reduction in the number of descending fibers, leads to the quantitative reduction in the development of the ventral nerve roots. In amphibia, the muscle segments on the side of the neural tube show evidence of a gradient, in which the high point is in the center and the activating rate grades off forwards and backwards. The existence of the gradient is expressed by pigment formed most abundantly in the region of high activity. In the developing muscle segments, the pigment is usually near the center, therefore, the septa between the segments are regions of low activity. Those peripheral

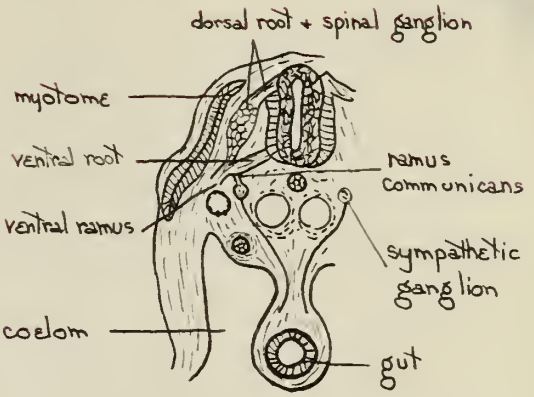
nerve fibers which emerge in the ventral region of the cord continuing to grow along a gradient (and eventually becoming axones) will accordingly grow to the center of each muscle segment and innervate it. On the other hand, regions of the septa, between the muscle segments, will attract dendrites of the sensory neurones which will then grow to the ends of the muscle segments (thus providing their proprioceptive innervation) and continue in the septum between the muscle segments to the skin. (Huxley and DeBeer, 1934). Bardeen, 1900, said that the first root fibers from the cord and ganglia of the pig grow out naked into the surrounding mesenchyme. Then, as the sensory and motor fibers intermingle they begin growth towards the myotome. Many long spindle cells appear among the developing nerve fibers which collect into bundles and form partial sheaths. A single bundle, slightly thicker than the rest and more completely ensheathed, runs ahead to direct the course of the nerve. Therefore, the nearer the nerve is to the spinal cord, the greater the number of bundles comprising that nerve. The nerve does not grow directly into the myotome because this is prevented by a dense layer of connective tissue which unites the scleromeres between the nerve and the myotome. Accordingly, the nerve is forced to the proximal side of the segment by the scleromere at the distal side. The nerve grows very rapidly and loose tissue grows in. As the

definitive muscles are formed, the loose mesenchyme of the muscle mass is an opportune spot for nerve growth.

Each spinal nerve typically has a dorsal, sensory root, and a ventral, motor root.[1] The fibers of the ventral root arise as a peripheral outgrowth from the neuroblasts situated in the mantle zone of the basal plate of the neural tube. (Fig. XIV) The dorsal root fibers come from the neuroblasts in the spinal ganglion which was derived from the segmentation of the primitive neural crest. The two roots then join to form a common trunk, the main trunk of the spinal nerve, which branches into three rami, (Fig. XIV). The ramus dorsalis terminates in the muscles and the sensory end organs in the dorsal part of the body. The ramus communicans ends in the sympathetic ganglionic chain lateral to the aorta. The third branch, the ramus lateralis, divides into the lateral and ventral rami. The former goes to the end organs of the lateral surface of the body, and the latter goes to the end organs of the ventral wall of the body. The main trunks of the spinal nerves are segmentally arranged corresponding to the myotomes. In those places where the mesodermal segments are crowded, the nerve trunks unite to form a plexus before dividing into the ventral and lateral rami. Therefore, the number of nerves in a

[1]The following discussion on the nervous system is based chiefly on the work of Jordan and Kindred, 1926.





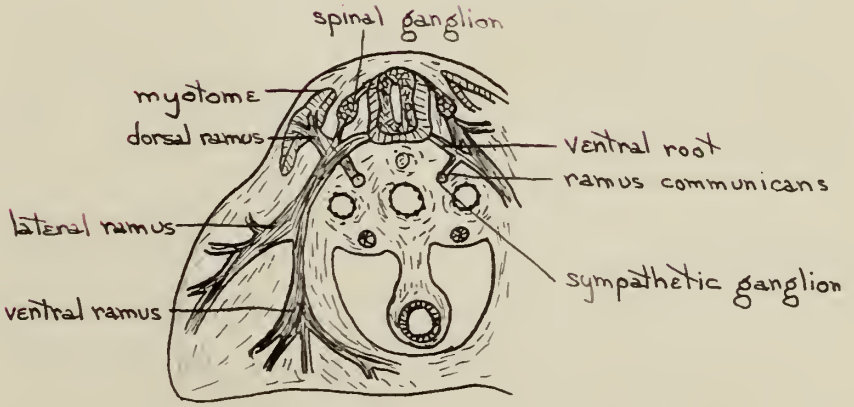


Figure XIV

Schematic Diagrams Representing the Development of a Spinal Nerve

(from Jordan + Kindred after Patterson + Cunningham)

plexus is the same as the number of somites contributing to the muscle and skeletal part supplied by that plexus.

Cerebral Nerves: None of the twelve cerebral nerves contain all four elements, somatic, motor, visceral motor and visceral sensory, and accordingly may be classified as follows:

A. Special Sensory: olfactory, optic, acoustic

B. Somatic motor: oculomotor, trochlear, abducens, hypoglossus

C. Visceral (mixed sensory and motor): trigeminal, facial, glossopharyngeal, vagus, spinal accessory.

A. Special sensory nerves: The olfactory (I) nerve is purely sensory. The optic nerve (II) is derived from the neuroblasts in the mantle zone of the retinal portion of the optic cup. The acoustic or auditory (VIII) develops from neuroblasts which differentiate in a ganglionic complex lying cephalomedial to the optic vessel, and carries impulses from the optic organs of equilibration and hearing.

B. The Somatic Motor nerves: Three go to the intrinsic muscles of the eye and the hypoglossal goes to the tongue. They originate in the gray matter of the basal plate of the brain and are homologous with the region of origin of the motor roots of the spinal nerves. The hypoglossal nerve (XII) innervates the thyroid muscles, and is

considered to be composed of several spinal nerves which have lost their dorsal roots. The ganglion in connection with the most caudal root atrophies early. The oculomotor nerve (III) which originates from the neuroblasts in the mantle layer of the basal plate of the mesencephalon, innervates the inferior oblique, and superior, inferior, and internal recti. The trochlear nerve (IV) arises just caudal to the oculomotor and enters the superior oblique eye muscle. The abducens (VI) arises cephalic to the hypoglossal in the myelencephalon and enters the mesenchyme primordium of the external rectus.

C. The Visceral nerves (mixed): The motor roots of the visceral cerebral nerves arise from a column of neuroblasts which are a cephalic continuation of neuroblasts of the lateral horn of the gray matter of the cord. The sensory fibers come from ganglia of neural crest origin and enter the neural tube to form the tractus solitarius. The facial (VII), motor fibers predominating, arises from a group of neuroblasts in the basal plate of the myelencephalon and terminates in the mesenchymatous tissue of the hyoid arch which is the primordium of the muscles of expression, and eventually the sense organs of the tongue. The glossopharyngeal (IX) is largely sensory and originates from primordial ganglia from the neural crest masses of the head. It forms two ganglia, a root ganglion and the petrosal ganglion. The fibers from these enter the brain caudal to the eighth nerve

and join the tractus solitarius. The peripheral fibers are grouped in two branches, one, the ramus tympanicus goes to the second branchial arch, and the ramus lingualis goes to the third. The few motor fibers arise from neuroblasts in the basal plate of the myelencephalon and terminate in the primordia of the muscles of the pharynx. The vagus complex, (X & XI). Some fibers of the spinal accessory arise from neuroblasts in the ventral horn of the spinal cord as far posterior as the fourth cervical segment, and others from the nucleus ambiquus of the myelencephalon and end in the mesenchymatous primordia of the sterno-cleido-mastoid and trapezius. Other motor fibers of the vagus end in the pharyngeal and laryngeal muscles. The sensory elements are from the ganglionic crest, a continuation from the neural crest of the head region. The trigeminal (V) has an extremely large ganglion, the Gasserian ganglion, derived from the most cephalic end of the neural crest. It sends fibers centrally to enter the pontal region of the myelencephalon. The peripheral fibers form the ophthalmic, maxillary and mandibular rami, terminating in the optic stalk, and the maxillary and mandibular processes respectively, of the first branchial arch. The terminations are eventually connected with the sensory end organs of the face, head, oral cavity, and teeth. The motor root arises from neuroblasts in the dorso-lateral portion of the basal plate of the

myelencephalon and terminate in the mesenchymatous primordia of the muscle of mastication.

The Autonomic Nervous System: The ganglia of the autonomic nervous system arise from cells which migrate from the cerebro-spinal ganglia and neural tube to their definitive positions. (Fig. XV). This system is usually subdivided into three parts, cranial, thoraco-lumbar and sacral autonomic systems. The thoraco-lumbar autonomic is commonly called the sympathetic nervous system and its preganglionic fibers emerge from the thoracic and upper lumbar spinal nerves. Likewise the cranial parasympathetic fibers system emerge from the third, seventh, ninth, tenth and eleventh cranial nerves, and the sacral parasympathetic fibers from the second, third and fourth sacral nerves. Ganglia of the trunk and viscera arise earlier in development than those of the head. In the sympathetic region, ganglion cells migrate before the formation of definitive nerve fibers, and in the head they follow the paths of the cerebral nerves arising from cerebral ganglia. During migration, cells are in an indifferent stage and only later develop into either neuroblasts or supporting elements. The neuroblasts sprout axones and dendrites as do the neuroblasts of the spinal nerves but these fibers do not become myelinated. In the trunk, the sympathetic ganglia are connected with the cerebro-spinal system by the rami communicantes and with

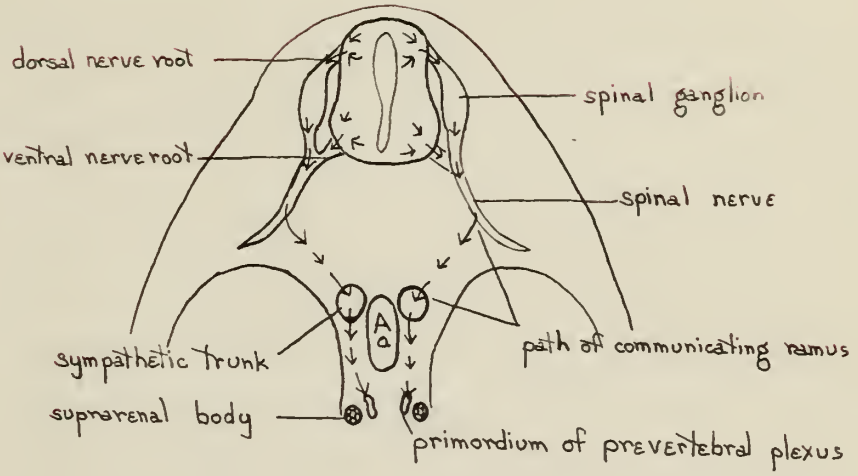


Figure XV

Diagrammatic Transverse Section of 9 mm. Embryo
(from Jordan & Kindred after Kuntz)

arrows indicate the course and direction of the cells migrating from neural tube and spinal ganglia into the sympathetic primordia.

each other by transverse (caudally) and longitudinal trunks. In the head it is the same except the latter connections are missing.

The primordia of the sympathetic ganglia are first seen in five millimeter embryos as groups of cells extending downwards from their origin from the cerebro-spinal ganglia and neural tube to the region dorsal-lateral to the aorta, and here they form continuous cellular cords on either side of the aorta. Later cells have been observed migrating from the wall of the tube near the ventral root of the spinal nerves and it is thought that they eventually reach the cellular cords formed by other migrating cells. The cord of cells segments and definitive ganglionic masses appear. The nerve fibers grow into these masses from neuroblasts of cord and spinal ganglia, forming the rami communicantes. Later nerve fibers of the neuroblasts in the sympathetic ganglia grow centripetally and communicate with the central nervous system, remaining unmyelinated and are the gray rami of the rami communicantes. Fibers grow peripherally from these neuroblasts to the smooth muscle of the viscera and vasa, and to the glands of the viscera. These fibers are largely efferent and the cell bodies are in contact with the axones of spinal motor neurones which enter the autonomic ganglia from the spinal cord. Other autonomic cells develop fibers which extend longitudinally forming fibrous components of

the longitudinal autonomic trunks. The prevertebral ganglia (celiac and hypogastric) and plexuses of the hinder part of the intestine are formed from cells which continue to migrate beyond the sympathetic ganglia and form ganglionic masses in a region ventral to the aorta and in the intestinal wall. (Fig. XV). The autonomic ganglia of the heart, lungs and anterior portion of the alimentary canal arise from cells which migrate along the paths of the vagus nerve from the vagus ganglia and wall of the neural tube.

As early as 1909, Boeke, studying skeletal muscle, recognized the existence of fine unmyelinated nerve fibers which he later placed as accessory fibers belonging to the autonomic nervous system. He placed those that he found in the eye muscles as part of the cranial parasympathetic system. This work of the existence of autonomic fibers in skeletal muscle was immediately taken up by many others, some of whom, Hines and Tower, (1928), Wilkinson, (1929), and Coates and Tiegs, (1931) failed to corroborate the findings of Boeke. Others who agreed with him, particularly Kuntz, carried on series of experiments on cats, dogs, pigeons, frogs and other animals to study the presence of these nerve fibers as they affected the muscle tonus and muscle fatigue. From the work of Kuntz, (1934), which is a compilation of the study of other investigators, the presence of autonomic nerve fibers in skeletal muscle is

considered by many present biologists as an hypothesis which still lacks adequate proof.

The investigations of those who claim the presence of these fibers indicates that the sympathetic fibers in the muscles are conveyed peripheralwards in the cerebrospinal nerves and are close to the blood vessels. The existence of parasympathetic nerve supply to the skeletal muscle is not as conclusive as the sympathetic innervation, but the work of Hinsey, (1927), Imagawa, (1927), Sunaga, (1927) and others maintains that such innervation exists.

From the results of many experiments on the action of the autonomic nerve fibers, Kuntz, (1934), says that much of the influence on skeletal muscles is expressed through its effect on muscle metabolism which is brought about in part through the direct effect of autonomic impulses on the muscle cells. However, he continues to say that the result of many of the experiments must be considered inconclusive, although the direct effect on the muscle tonus, fatigue and stimulation seems to be demonstrated to some extent.

For its purpose in this paper, it will be suffice to say that recent work with the skeletal muscles of various animals including the bird indicates that these muscles are also innervated by the autonomic nerves, but so far as is known, these nerves have nothing to do with the actual development of the muscles.

SUMMARY

1. The purpose of this paper was to bring together in one paper the beliefs and experimental results of the investigators in the developing musculature of the chick; treating the earliest embryologists from an historical point of view and concentrating the bulk of this account on the theories and experiments carried on in the twentieth century.

2. All embryologists agree that muscle tissue, with exception of some in the eye region and dermal muscle, is of mesodermic origin. The bulk of the musculature is derived from the myotome portion of the somites.

3. The mesoderm appears soon after the twelfth hour of incubation and rapidly extends between the ectoderm and the entoderm, and is noticeably thicker on either side of the mid-line of the embryo than it is further laterad.

4. Segmentation of the mesoderm begins as a V-shaped transverse fissure in the thickened mesoderm of the vertebral plate midway between the anterior extremity of the embryo and the primitive streak. Each succeeding fissure develops posterior to those already formed, and the process of segmentation or somite formation progresses posteriorly. The first formed somite therefore is also the most anterior one.

5. Each somite first appears as a solid mass of cells,

and then a cavity is formed which is soon filled with a core of cells that are epitheloid and syncytial in character. On the ventro-mesial surface the core and outer portion merge to form the sclerotome. The dorsal portion of the outer surface becomes the dermatome, and the medial wall just below becomes the myotome.

6. Two types of muscle tissue are formed, smooth and cross-striated. Smooth muscle at first consists of condensed mesenchyme with elongated nuclei which is followed by the formation of myofibrillae, coarse and fine. The coarse myofibrillae lose their granular appearance and become varicose and smooth. Later fine myofibrillae from the embryonal connective tissue arise directly as such. Cross-striated muscle develops in a similar way but is derived only from myoblasts. Fibrillae develop which extend from one myotome to another, and show the Q and I bands. Degeneration of some of the fibrillae also occurs and the new fibrillae are formed by longitudinal splitting of the normal ones already present.

7. The skeletal muscles, except those from the branchial arch mesoderm, originate from the myotomes. Although their primitive segmentation is lost, the original innervation by the segmental nerves is retained.

8. The process of transformation of the premuscle mass into the definitive muscles is as follows:

(a) A change in direction of the muscle fibers from their original cranio-caudal orientation in the myotome. The fibers of but few muscles retain this initial orientation. Such persistent muscles give rise to the deep spinal muscles.

(b) A migration of myotomes, wholly or in part, to more or less remote regions. Thus the latissimus dorsi originates from cervical myotomes but finally attaches to the lower thoracic and lumbar vertebrae and to the crest of the ilium. Other examples are the serratus anterior and the trapezius.

(c) A fusion of portions of successive myotomes. The rectus abdominis illustrates this process.

(d) A longitudinal splitting of myotomes into several portions. Examples are found in the sterno- and omohyoid and in the trapezius and sterno-mastoid.

(e) A tangential splitting into two or more layers. The oblique and the transverse muscles of the abdomen are formed by this common process.

(f) A degeneration of fibrillae. In this way fascias, ligaments, and aponeuroses may be produced.

9. There is considerable dispute concerning the origin of the limb muscles. According to some writers, the principal and only source of limb muscle is from the undifferentiated, diffuse mesenchyme which forms the core of the limb

bud. Other writers believe that the myotome plays some part, perhaps only to the extent of contributing a few migrating cells.

10. The muscles of the head develop from cells of the mesoderm of the branchial arches which become detached and migrate cephalad and also from muscle tissue arising from ectoderm in the region of the eye.

11. The early innervation is discussed because of its close relationship with the developing muscle, and because of its significance in tracing the origin of muscles. Early muscle development, however, is independent of nervous influence.

BIBLIOGRAPHY

BARDEEN, CHARLES R. The development of the musculature of the body wall in the pig, etc. Johns Hopkins Hosp. Rep., Vol. 9, 1900, pages 367-399.

BURROWS, M. T. The growth of tissues of the chick embryo outside the animal body, with special reference to the nervous system. Jour. Exp. Zoo., Vol. 10, 1911.

BYRNES, ESTHER F. Experimental studies on the development of the limb muscles in Amphibia. Jour. Morph., Vol. 14, June 1898.

CAREY, E. J. Direct observations on the transformation of the mesenchyme in the thigh. of the pig embryo (sus scrofa) with especial reference to the genesis of the thigh muscles, of the knee and hip-joints, and of the primary bone of the femur. Jour. Morph., Vol. 37, 1921.

CAREY, EBEN J. Studies in the Dynamics of Histogenesis. IV Tension of Differential Growth as a Stimulus to Myogenesis in the Limb. V Compression between the Accelerated Growth centers of the Segmental Skeleton as a Stimulus to Joint Formation. VI Resistances to Skeletal Growth as Stimuli to Chondrogenesis and Osteogenesis. Am. Jour. Anat., Vol. 29, p. 93, 1921.

CAREY, EBEN J. Studies in the Dynamics of Histogenesis. Tension of Differential Growth as a Stimulus to Myogenesis. VII The experimental transformation of the smooth bladder muscle of the dog, histologically into cross-striated muscle and physiologically into an organ manifesting rhythmicality. Am. Jour. Anat., Vol. 29, p. 341, 1921.

DALTON, A. J. The potencies of portions of young chick blastoderms as tested in chorio-allantoic grafts. Jour. Exp. Zoo., 71 (1), 1935.

EDGEWORTH, F. H. Development of the head muscles in Gallus domesticus and the morphology of the head muscles in the Sauropsida. Quart. J. Micri. Sci., new series, Vol. 51, 1907, pages 511-556.

FOSTER & BALFOUR. The MacMillan Co., New York. The Elements of Embryology, 1910.

GLADSTONE, R. J. and COLWELL, HECTOR A. On some effects of X-rays on developing chick embryos. Jour. Anat., 68 (1), 1933, pages 85-95.

HARRISON, R. G. On the differentiation of the muscular tissues when removed from the influence of the nervous system. Am. Jour. Anat., Vol. 2, 1905.

HARRISON, R. G. An experimental study of the relation of the nervous system to the developing musculature in the embryo of the frog. Am. Jour. Anat., Vol. 3, 1906.

HOADLEY, L. Differentiation of isolated chick primordia in chorio-allantoic grafts. II The effect of the presence of the spinal cord, i.e., innervation, on the differentiation of the somitic region. Jour. Exp. Zoo., 42, 1925.

HOADLEY, L. The in situ development of sectioned chick blastoderm. Arch. de Bid., T 36, p. 225.

HOOKER, D. The development and function of voluntary and cardiac muscle in embryos without nerves. Jour. Exp. Zool., Vol. 11, pages 159-187, 1911.

HUNT, E. A. Differentiation of chick limb bud grafts with special reference to the muscles. Jour. Exp. Zoo., 62, 1932.

HUNTER, RUSSELL P. The early development of the hypoglossal musculature in the chick. Jour. Morph., 57 (2), 1935, pages 473-499.

HUXLEY & DEBEER. University Press, Cambridge (Eng.). The Elements of Exp. Embryology, 1934.

HYMAN, L. H. The metabolic gradients of vertebrate embryos--the chick. Biol. Bull., 52, pages 1-39.

JORDAN & KINDRED. D. Appleton & Co., New York. A Textbook of Embryology, 1926.

KRASSOUSKA, O. V. Differentiation of skeletogenic mesenchyme of chick embryo in vitro. Arch. Sci. Biol. (Leningrad), 34 ("3), 1934, pages 193-208. Biological Abstracts.

KUNTZ, ALBERT. Lea & Febiger, Philadelphia. The Autonomic Nervous System, 2nd Edition, 1934.

LEWIS, W. H. The development of the muscular system. Manual of Human Embryology. Keibel & Mall, Vol. 1, 1910.

LILLIE, FRANK R. Henry Holt & Co., New York. The Development of the Chick, an Introduction to Embryology, Sec. Ed., Revised, 1919.

LONG, J. A. Dissection as a method of embryological study. Science, 80 (2086), 1934, p. 592.

MCEWEN, ROBERTS. Henry Holt & Co., New York. Vertebrate Embryology, 1923.

MINOT, C. S. P. Blakiston's Son & Co., Philadelphia. Laboratory Textbook of Embryology, Sec. Ed., 1911.

PATON, STEWART. Experiments on Developing Chicken's Eggs. Jour. Exp. Zoo., Vol. 11, p. 469, 1911.

PATTEN, BRADLEY M. P. Blakiston's Son & Co., Inc., Philadelphia. The Early Embryology of The Chick, Third Edition, 1929.

PATTERSON, A. M. On the fate of the muscle plate and the development of the spinal nerves and limb plexuses in birds and mammals. Quart. Jour. Mic. Sci., Vol. 28, pages 109-130, 1888.

PATTERSON, T. J. The order of appearance of the anterior somites of the chick. Biol. Bull., Vol. 13, pages 121-133, 1907.

PRENTISS, CHAS. W. & AREY, LESLIE B. W. B. Saunders Co., Philadelphia. A Laboratory Manual & Textbook of Embryology, Second Edition, 1917.

RAWLES, MARY E. A study in the localization of organ forming areas in the chick blastoderm of the head process stage. Jour. Exp. Biol., 72 (2), 1936, pages 271-315.

ROMANOFF, A. L. Biochemistry and Biophysics of the Developing Hen's Egg. I Influence of Humidity. Cornell University Aggie Bull., 132, Aug. 1930.

ROMANOFF, A. L. & FABER, H. A. Effect of Temperature on the Growth, Fat, Calcium Metabolism, Mortality of the Chick Embryo during the latter part of Incubation. J. Cell & Comp. Physiol., Vol. 2, Feb. 1936.

ROMANOFF, ALEXIS L. Cultivation of the Chick Embryo in an Opened Egg. Anatomical Record, Vol. 48, Jan. 1931.

ROMER, A. S. The development of the thigh musculature of the chick. Jour. Morph. & Physiol., Vol. 43, pages 347-385, 1927.

RUDNICK, DOROTHEA. Regional restriction of potencies in chick during embryogenesis. Jour. Exp. Biol., 71 (1), 1935, pages 85-99.

SPURLING, ROY G. The effect of extirpation of the posterior limb bud on the development of the limb and pelvic girdle in chick embryos. Anat. Rec., Vol. 26, pages 41-56, 1923.

WADDINGTON, C. H. Experiment on embryonic induction. I. The competence of the extra embryonic ectoderm in the chick. Jour. Exp. Biol., 11 (3), 1934, pages 211-227.

WALTERS, NELSON F. Changes in position of chick embryos after 18th day of incubation. Science, 82 (2116), 1935, pages 66-67.

WILLIAMS, L. W. The somites of the chick. Am. Jour. Anat., Vol. 11, pages 55-100, 1910.

WINDLE, W. F.; MINEAR, W. L.; AUSTIN, M. F.; & ORR, D. W. The origin and early development of somatic behavior in the albino rat. Physiol. Zoo., 8 (2), 1935, pages 156-185.

WINDLE, W. F. & ORR, D. W. The development of behavior in chick embryos: Spinal cord structure correlated with early somatic motility. Jour. Comp. Neurol., 60 (2), 1934, pages 282-307.

BOSTON UNIVERSITY
1 1719 02557 0203